

Guidelines for helping industry

The present clamour that British academics should do more to help British industry is understandable but, so far, also unreflective. Everybody's interest would be helped by explicit guidelines.

THE British Government's advocacy during the past two years of closer links between higher education (universities in particular) and industry has had at least one important consequence — never before have so many committees deliberated on the question. Last month (see *Nature* 30 June, p.743), a committee of the Advisory Council on Applied Research and Development (ACARD) reported on its deliberations, recommending that closer working between these two partners should be stimulated by a blend of exhortation (fatuously, by "bodies on which industry is represented at a high level") and financial incentives (to be offered to universities and polytechnics by the Department of Industry). More recently, the chairmen of ACARD and of its academic *doppelganger*, the Advisory Board for the Research Councils (ABRC), have issued what promises to be the first of an annual series of commentaries on British research which echoes the views of the two bodies on what needs more urgently to be done — more industrial collaboration (ACARD) and a greater proportion of the university recurrent budget spent on research (ABRC). As if not to be outdone, the University Grants Committee has now also chipped in with its shockingly (but customarily) delayed report on the events of 1981–82, offering its special support for biotechnology as an illustration of what it can do for industry and saying that it has asked the universities what they plan to do to maintain the research effort from their recurrent budgets. What will the government make of all this?

Most probably, very little. Although the ACARD/ABRC document urges the need for selectivity in public spending on both academic and industrial research, the combined effect of the documents is hopelessly unselective in its advice. The danger now is that ministers anxious to foster relations between universities and industry will be left with the impression that there is a great opportunity beckoning, but no sure way in which it can be won. Perhaps the authors of these reports can use the occasion when most of them are speaking at a conference at Imperial College London on 21–22 July to make plain just what advice they meant to offer. Meanwhile, they should consider the important matters hitherto overlooked in the new-found fondness for collaboration between universities and industry.

The reasons why links between higher education and British industry have been only meagre need to be recognized more openly. (The ACARD panel estimates that higher education earns only £40 million a year from its industrial relationships, but this is either very large or very small according to one's expectations.) First, it is not true, as much of this rhetoric suggests, that British higher education and the universities in particular are slothfully indifferent to the need for national prosperity. This view ignores

the structural impediments to collaboration. Until the great budget cuts in 1981, the genteel umbrella of the University Grants Committee worked for uniformity, with the result that grant-making organizations such as the research councils were forever seeking special schemes to encourage links between academics and industrial companies. Only now that some universities have recognized that their survival will ultimately depend on what they can do to save themselves has the search for industrial support gathered momentum. Universities such as Salford (see *Nature* 2 June, p.370) have explicitly abandoned the Oxbridge ideal (and would say that they have never held to it) and are instead trying to emulate Imperial College. And, now, the pressure on others to follow suit is growing. What the British Government needs to understand is that such pressure is mistaken. What British higher education needs is diversity, not uniformity. And the practice of diversity requires an even greater degree of intellectual autonomy than at present, dependable foreknowledge of what the government will pay towards recurrent costs and freedom from the inequitable and iniquitous practice (as in the past two years) of regulating universities by limiting the numbers of students they may educate. Within such a framework, some universities would no doubt choose to concentrate on the academic (not a synonym for "useless") research from which they have already made striking reputations, while others would forge links with industry not merely for the money but because practical achievement is also fun. Those ministers, members of parliament and officials who talk as if academic research is akin to treachery at times of such economic crisis should be made to understand that the education of students (in science as well as technology) is also relevant and important.

Lost opportunities

Second, it must be recognized that a great deal (and not merely exhortation) needs to be done to change the industrial climate in which British higher education functions. Both industry and the government have been slow to appreciate that universities have anything worthwhile to offer industry, which squares only poorly with the common lament that British universities are prolific sources of ideas afterwards exploited elsewhere (penicillin, digital computers, etc.). But if such complaints are true, who but British industry can be held at fault? Further evidence in this direction is provided by the relatively poor financial rewards now offered to people willing to become industrial scientists, the dependence of many basic industries in Britain (chemicals and pharmaceuticals are honourable exceptions) on research subventions from government and the common assumption that the obligation of higher education to help foster industrial well-being should not extend to managerial responsibility for substantial projects (on the grounds that academics do not understand the need for deadlines). The plain truth is that many of the British industries most in need of technological help do not recognize the need. The ACARD panel's suggestion that the government should use its purchasing power outside the defence field to encourage industrial collaboration with higher education is a dubious remedy (which was tried in the 1960s), dependent as it is on the capacity of civil servants and their advisers to act as entrepreneurs by proxy. Why not, instead, make it easier for true entrepreneurs to succeed, and for established but inefficient businesses to fail?

DNA now and tomorrow

AT the conference to celebrate the 30th anniversary of the publication of the structure of DNA to be held in Boston on 19–21 September 1983, up to 50 bursaries will be offered to young scientists working in the field. The registration fee will be waived for successful applicants, but they will have to meet other costs themselves. Applications should be marked "Bursary" and sent to the Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF by Friday 12 August. □



The third set of problems too commonly overlooked concerns the degree to which the proper role of higher education in industrial communities such as Britain may be compromised by too headlong a pursuit of industrial liaisons. The ACARD report acknowledges some of these difficulties, but only to dismiss them. Impediments to free publication? "We feel it reasonable that the provider of funds should control the publication of results . . ." Applied research might dominate basic research? " . . . The current emphasis is the other way." Fears that one company may benefit at the expense of others? " . . . The return to the nation for its involvement in publicly sponsored research" comes from the taxes paid by the companies concerned, and from the employment their business create. The conflict between the pursuit of scholarship and the concerns of industry? "Such attitudes, where they exist, reflect the way in which industry and the academic world have failed to come together in the United Kingdom . . ." These questions and answers overlook the seriousness of the problems that British universities need to contend with if (as is to be hoped) they find ways of contributing more directly to industrial innovation, and that now need to be examined closely not merely as academic issues but in the light of often painful experience elsewhere, in the United States for example.

Whatever ACARD says, the stampede for more industrial links with higher education will compromise the impartiality of the university as a source of advice to all comers, and safeguards need to be devised. The fact that a company enjoying a successful relationship with some university may in due course pay higher taxes will be little comfort to some other company denied access to the same source of assistance, while exclusivity as such is unlikely in the long run to benefit the British economy. Unfortunately, no one set of guidelines will cover all circumstances. Academic scientists working as consultants for private companies should be required to disclose their relationships not only to their colleagues but, if asked, to other companies. Academic innovators with something to patent should share the proceeds with their institutions, which should have responsibility for making sure that the patent goes to the company offering the best terms (but should not seek patent protection themselves). Academics and institutions carrying out projects under contracts with industrial organizations should ensure that the full costs (overheads as well as direct costs) are properly covered, and should be prepared to welcome supervision by the University Grants Committee to ensure that public funds are not thus used indirectly to support private enterprises. Much of this will seem needlessly irksome, yet explicit regulations of this kind are a necessary means of making sure that industrial liaisons prosper.

It is just as important that the principal function of higher education — the education of students — should not be compromised by the flourishing of industrial liaisons. The central issue is whether the universities, other institutions and their academics will be distracted from their central role by the search for extra revenue, marginal though it will usually be. Most institutions are aware of the danger, and on their guard against the obvious risks. There is, however, a hidden risk that needs attention. One of the ways in which the British economy is deficient in comparison with its competitors is that too few young people elect to pursue technical courses of study. There is ample evidence that both the proportions following technical courses and their enthusiasm for them are restricted by the demands at secondary schools for early specialization. (Only this week, a body called the Secondary Science Curriculum Review published its plan for changing all that.) The result is that the demand for technical education in higher education is less than would naturally be the case. In short, a more sensible way of introducing the school population to what science is and is about would dramatically increase demand for higher education in this field but also occasion structural changes. How, the universities should be asking themselves, would they be able to live with such a development, itself the best thing that could happen to British industry, and at the same time serve as prime movers in the process of industrial innovation? That, mercifully, is a question that cannot for much longer be put off. □

Unthinkable abilities

The death of Herman Kahn is a sad loss to realism in international affairs.

HERMAN Kahn, who died last week, was an enormous man, physically and in intellectual stamina. He was also one who deliberately provoked a reputation as an *enfant terrible*, and whose self-appointed role was to challenge the accepted beliefs of the liberal establishment to which, after the Second World War, and with his background at the California Institute of Technology, he might have been expected to belong. Instead, Kahn became a Rand Corporation man and then, with *On Thermonuclear War* (published in 1960 in a lurid red and yellow jacket) one who was commonly supposed to advocate nuclear warfare as a routine instrument of international relations. Kahn, of course, had said nothing of the kind — merely that those who pinned their faith in deterrence by nuclear weapons as an instrument of strategy should at least contemplate what might happen if they were compelled by their strategic doctrine to make use of them. His advocacy of building shelters for at least a proportion of the population was similarly understood to be a case for saying that nuclear warfare could be winnable in some absolute sense — a misunderstanding in retrospect understandable because of the density of Kahn's prose and because he set out arguments in note form, as if he were about to give a lecture studded with shocking aphorisms. The storm provoked by this book, which might have been more influential if it had been more subtle (but which might also then have become yet another treatise among a flood of others at that time), left Kahn unabashed. He went on to invent the phrase "think the unthinkable", the activity that became his stock in trade.

Kahn was in reality not the ogre public legend required. Surprisingly, he was also one of those congenial optimists given to bouts of depression by manifestations of the disbelief of others. This is the spirit in which he found it hard to come to terms with the widespread belief, common in the United States fifteen years ago, that economic growth must somehow be wicked and, a little later, the widespread indifference to the emergence of Japan as an industrial power. Kahn's perception of his role was that of a prophet most often ignored until too late.

Part of the trouble was his own impatience with conventional means of communication. Although capable of a fluent 55-minute talk on virtually any subject at the drop of a hat, he seemed curiously indifferent to the importance of verbs in written sentences, or the need to connect one sentence with the next. His style was that of one who had assembled numerical evidence from various unlikely places, who had perceived some conclusion buried in the data and who could not for the life of him understand why the same should not be true for his readers. But Kahn was often overwhelmed by the speed with which his own ideas came tumbling out, so that sentences had to be left unfinished because the next had already begun, and because he had succeeded in applying the vocabulary of lunch-room physics to international relations and national security. Even in later life, Kahn seemed to have kept the habits of a precocious but still not educated student whose more sober teachers whisper to each other that he may be too smart by half.

Kahn's most obvious legacy is the Hudson Institute in New York State, always more boisterous than the Rand Institute (and much smaller). Being so obviously Kahn's own thing, the institute may have some uncomfortable times ahead. But even for those who profoundly disagreed with him, Kahn's chief interest is that he preached what he considered to be realism at a time when realism was out of fashion. Kahn's unthinkables were not, after all, beyond the scope of thought but were merely outlawed by convention. It is admirable that a man so burdened by being overweight, and whose visits to various cities often took the form of checking into the nearest comfortable hotel to the airport and there holding court should have stirred up so much thought. Even Kahn's opponents will acknowledge that. □

Clinch River

Breeder reactor to breed more bonds

Washington

IN a final effort to save the Clinch River breeder reactor, the electric utility industry has formulated a plan to distribute the project's \$3,500 million construction costs more evenly between the government and the private sector. Under the plan, submitted earlier this month to the Department of Energy (DoE), \$1,000 million would be raised through the sale of bonds to private investors; this would represent some 40 per cent of the money needed to complete the reactor.

Last month, a coalition of nuclear critics and fiscal conservatives in Congress finally achieved what they have for several years come within a hair's breadth of doing: deleting funds for Clinch River. What tipped the scales was not concern over safety or even nuclear proliferation (which former President Carter made the central issue in suspending work on the breeder and on reprocessing of plutonium), but the increasingly skewed economics of what was to have been an industry-government partnership. Under the original contract, signed in 1972, industry and government were each to have borne half of the costs, then estimated at \$500 million. But the contract specified that all overruns were to be borne by the government alone. Industry's \$257 million share, originally 50 per cent of the total cost, fell to below 10 per cent.

In failing to appropriate any funds for the project for 1984, Congress did not quite kill it. It had earlier indicated that if the administration presented a realistic proposal for redistributing costs, it might be willing to go ahead and approve funds. But that would have to occur before the beginning of the new fiscal year on 1 October. DoE is expected to act quickly on the utilities' new plan, and may bring it before Congress as early as next month.

The plan does not actually call on the utilities to spend any more of their own money. Instead, it would use the industry's contribution (\$150 million so far, plus an additional \$175 million to come) and a government guarantee of revenues from the sale of electricity from the plant to back the sale of \$1,000 million in bonds. The bonds would be backed by an indirect contribution from the government of a further \$150 million in tax credits and depreciation benefits. The utilities say they have been assured by several leading investment houses that the bonds, so backed, would be competitive in the financial markets. Wallace Behnke, chairman of the Project Management Corporation — the consortium of utility contributors to the project — said there seemed to be little possibility of the utilities' obtaining approval from the

state rate commissions for increasing their direct contributions to the project. (As state-regulated monopolies, the utilities must clear all such financial decisions with the state commissions.)

Although \$1,000 million has already been spent on Clinch River, construction has been repeatedly delayed. Following the

UK nutrition

Government chokes on report

WHOLEFOOD enthusiasts may have been right all along. That seems to be the message of nutritional guidelines contained in a draft report from NACNE, the National Advisory Committee on Nutrition Education. However, the Department of Health and Social Security (DHSS), which established NACNE to provide expert advice for health educators and government, appears to find the conclusions unpalatable. The report was first drafted more than two years ago but DHSS has so far refused to publish the guidelines, as the committee intended. The latest draft is described by one disillusioned scientist as "an emasculated version" of the original.

The existence of the report was disclosed in last week's *Sunday Times*. It was prepared at NACNE's request by an *ad hoc* group of 10 nutritionists chaired by Professor Philip James, director of the Rowett Institute in Aberdeen. The group sets down a consensus view of an average diet aimed at maintenance of health and primary prevention of disease, particularly those of public health importance such as heart disease.

Unlike previous nutritional guidelines from DHSS, James's group puts figures on the proportions of different nutrients in what it considers a healthy diet. Recognizing that most nutrition-related diseases in the United Kingdom are "diseases of affluence", the emphasis is on lowering average intakes of certain diet components rather than defining minimum intakes: it advocates the term "healthy varied diet" to replace "balanced diet" in the health educator's phrase book.

Following the Royal College of Physicians, NACNE concludes that even mild degrees of obesity are important for public health, being associated with cardiovascular disease and diabetes. The desired reduction in average weight would best be achieved by decreasing the proportion of total energy supplied by dietary fat from 38 per cent to 30 per cent, with an increase in the ratio of polyunsaturated to saturated fat. Contrary to previous advice, NACNE recommends an increase in total carbo-

hydrate intake; sucrose intake, however, should be halved, to about 20 kg per head per year. Total energy intake would remain unchanged. Intake of dietary fibre, especially cereal fibre, should be increased by 50 per cent to 30 g per day for adults, and salt intake should be cut from 8–12 g per day to nearer 5 g per day. More fresh fruit and vegetables would prevent deficiencies of folic acid, vitamin C and (by increasing uptake) iron.

The group's report was welcomed by NACNE's chairman, Professor Jeremy Morris of the London School of Hygiene and Tropical Medicine. But, not surprisingly, it is unpopular with the food industry; manufacturers of sugar, salt and some dairy products may have good cause to be alarmed. The British Nutrition Foundation, an independent body of scientists and representatives of the food industry, has drawn attention to the "far-reaching economic implications worldwide" that would follow from substantial reductions in sugar or butter consumption.

The NACNE working group says its proposals could be achieved within 15 years; the agricultural and food industries will, it says, be able to make plans in good time for adjustments in processing and labelling. NACNE suggests a breeding programme to produce leaner meat and a reduction in the fat content of meat products. One third of the proposed changes could be implemented within five years.

A spokesman for DHSS says the latest version of the report (thought to be the ninth) will be reviewed by the Committee on Medical Aspects of Food Policy, known affectionately in the department as Coma. The department says that there is no obligation to publish the report, because the group that produced it was not a formally constituted subcommittee. One of the ironies in DHSS's reluctance is that, together with reports from the Royal College of Physicians and the World Health Organization, DHSS publications formed one of NACNE's main sources of information.

Stephen Budiansky

Tim Beardsley

Hepatitis B vaccine

Pasteur Institute in AIDS fracas

ACQUIRED immune deficiency syndrome — AIDS — is raising strong emotions in France, particularly at the Institut Pasteur in Paris. The institute is still perhaps the most prestigious institution in French biology, so there was strong reaction there to the newspaper headline "Institut Pasteur, sick with gay cancer" (*Libération*, 27 June). The supposed problem was not with the research institute itself, but with its independent commercial offshoot, Institut Pasteur Production (IPP) which was accused of clandestine importation of American blood plasma (automatically suspected of AIDS contamination) to help with manufacture of hepatitis B vaccine. A chimpanzee was also said to have died in testing the first batch of such vaccine: it was an apparent scandal.

So last week the Institut Pasteur proper came to the aid of its offspring, while still keeping a little distance from it. François Jacob, Nobel prize-winner and doyen of the Institut Pasteur, described the *Libération* headline as "scandal-mongering". *Libération* should also have distinguished between the Institut Pasteur and IPP, he said. (The institute has a 45 per cent shareholding, but little influence over IPP.) And in an official press statement, it was said that "The Institut Pasteur is used to criticism. But cannot accept slander."

IPP is itself about to sue *Libération* for FF1 million for defamation and for possible loss of business in a fierce competition with its American rival, Merck Sharp and Dohme. Both companies are seeking lucrative contracts in Asia, and particularly in China where IPP had foreseen a market of "dozens of millions of doses of vaccine", and order of magnitude larger than its present sales. IPP expected the vaccine soon to become its major product. However, IPP's market position may have been compromised by the press attacks, IPP research director M. Prunet said on Friday.

As for some of *Libération*'s accusations, the truth now seems a little difficult to establish since French Health officials who earlier were said to have been "furious" about not having been informed by IPP about the use of American plasma now have to accept a Ministry of Health statement that the ministry was, in fact, informed, and had granted authorization from the first date of importation in March 1982. It must be remembered, said Dr Jacques Netter, responsible for the National Health Laboratory which monitors testing of the IPP vaccine, that when the authorization was given, AIDS was not so important. The real question is whether the vaccine is good and safe, and it is.

On other matters, things are clearer. The chimpanzee was one of a colony treated with vaccine, on which a liver biopsy is done each month. In this particular healthy chimpanzee, treated with the first lot of

vaccine to be based in part on American plasma (3 per cent of the total), there was a small lesion of the liver. Two French and one American expert concluded it was "nonspecific" and the vaccine was marketed (some to West Germany) with the approval of the French Ministry of Health, says M. Prunet of IPP. However, there had been "some disagreement" (says Dr Netter) among the experts about the nature of the lesion. When a kit for detecting human T-cell leukaemia virus (HTLV) — a suspected AIDS agent — arrived from the United States, the ministry requested a new test. Marketing was stopped for a while but the test proved negative and sales were resumed.

Moreover, IPP's production process seems to be thoroughly safe, at least in the elimination of known infectious agents. In tests, viruses such as polio introduced deliberately into the initial plasma are reduced by factors of 10^{20} by centri-

fugation and chemical treatment (ending with formaldehyde), M. Prunet claims. Formaldehyde "denatures" nucleic acid, he says. Another, enzymatic test, detects reverse transcriptase, the signal for retroviruses (like HTLV). So if AIDS is due to a virus with properties biologists understand, the IPP process eliminates it, Prunet says. If it is not, he has "no answer". But no case of AIDS has yet resulted from IPP's use of the vaccine on over 10,000 patients, and a follow-up study with the similar Merck vaccine in the United States has shown it causes no incidence of AIDS in low AIDS-risk patients and no significant change (in fact, a slight decrease) in high risk patients.

Libération is left with one substantial point: that confusion over the origin of IPP's plasma, and an early lack of information about the chimpanzee, which resulted in the facts being "discovered" by journalists, indicate a lack of "clarity" in IPP's affairs; and that it would have been much better for the company if the confusion had not been allowed to arise. IPP might heartily agree. **Robert Walgate**

Whaling

Norway this year's villain?

THE outlook seems stormy for next week's annual meeting of the International Whaling Commission (IWC) in the English south-coast town of Brighton. Delegates from Norway, in particular, are steeling themselves for a difficult time. And at the back of everyone's mind is the question of what is likely to happen to last year's resolution that zero catch quotas should be imposed for all commercial whaling from the end of 1985.

After that resolution was passed last year, four countries (Japan, Norway, Peru and the Soviet Union) lodged formal objections and so are not bound by it. Japan considered pulling out of IWC altogether, but has not yet done so. IWC itself is powerless to enforce the resolution, so it will be up to individual countries to provide the teeth. Only the United States is bound to impose sanctions, but they will not be used until the resolution is actually infringed.

Norway's problems concern stocks of Minke whales in the North Atlantic. Last year the commission was unable to agree on a catch quota, but Norway diplomatically adopted a figure of 1,690. New evidence from IWC's scientific committee suggests that stocks are substantially lower than the Norwegians assumed, so there will be strong pressure this year for a very much smaller quota.

Minke whales are also taken in the Antarctic, by Japan and the Soviet Union. Last year a quota of 7,072 was agreed, based on estimates of stocks extrapolated from visual sightings. The sightings, however, are made only in a small part of the Minke's range, and the scientific com-

mittee's evidence this year includes a strong criticism of the extrapolation procedure; some scientists believe it significantly overestimates whale stocks.

There is likely to be another big argument over stocks of Bryde's whale off the coast of Peru. Peruvian delegates will be in an embarrassing position: last year they unilaterally increased their catch quotas but then failed to catch anything approaching the figure. El Nino, the South Pacific current that has been behaving oddly this year, may be used to provide an explanation. The Peruvians can in any case expect



full support from the Japanese delegation, since Peru's whaling effort is in large part run by Japan. This, together with Japanese proposals to build a whaling station in the Philippines, should ensure that Japan is not kept entirely out of the spotlight.

Subsistence whaling by Eskimos in Alaska has been the subject of much attention. Last year a quota of 45 Bowhead whales over three years was agreed, but US officials have been under severe pressure to increase this total. At this year's Brighton meeting, the United States will try to increase the quota but scientists are arguing for a total ban on catches of the Bowhead. **Tim Beardsley**

Formaldehyde cancer risk

Court not convinced of dangers

Washington

A YEAR-LONG ban on the use of urea-formaldehyde foam insulation in homes and schools is about to be disallowed by a federal Court of Appeals. Declaring that the Consumer Product Safety Commission's ban was "not good science", the court in April overruled the commission's action; unless a stay is granted this week pending a planned appeal to the Supreme Court, the court's decision will take effect immediately. Even if a stay is granted, it may merely postpone the inevitable, as the Supreme Court is considered unlikely to agree even to hear the appeal.

The Court of Appeals' unanimous and strongly-worded decision said the commission had failed to provide adequate data to prove that formaldehyde released by the insulation presented an "unreasonable risk" — the legal standard for rules issued by the commission. The court particularly criticized a cancer risk assessment based on a single study involving 240 rats and on exposure estimates derived from measurements made in houses whose occupants had complained of acute symptoms of formaldehyde exposure — rather than in a random sample of houses with the insulation.

The industry, which brought the suit, argued that "complaint" households had higher than typical formaldehyde concentrations, usually as a result of improper installation, a problem that could be corrected by regulations that stop far short of a total ban.

The commission first began studying the problem as a result of complaints from homeowners who had installed the insulation. Headaches, respiratory irritation and dizziness were the most common complaints. Air samples taken in these houses revealed an average formaldehyde concentration of 0.08 parts per million (p.p.m.), compared with 0.03 p.p.m. in houses without the insulation. While the commission was investigating the matter, preliminary results of the rat carcinogenicity study became available, adding to the evidence against formaldehyde. In this study, carried out by the Chemical Industry Institute of Toxicology, rats exposed to relatively high levels of formaldehyde (14 p.p.m.) developed nasal tumours.

If, as expected, the Supreme Court affirms the Court of Appeals' decision, the commission could go back and redraft its regulation. But at the very least, it would need to provide more extensive monitoring data and a more substantial argument of the risk posed. On the other hand, the court did acknowledge the acute effects as "a real problem". And it dismissed one favourite argument of the industry, namely the failure of epidemiological studies in humans to show any carcinogenic effects. "It is highly unlikely that studies involving

a total of 10,000 workers would detect such a small risk" of increased cancer as is at stake at the exposure levels involved, the court said.

The effect of the court's decision is somewhat dampened by the existence of state and local bans on urea-formaldehyde insulation that will remain in force, and by a now widespread reluctance on the part of homeowners to install the insulation. This reluctance was intensified by an "advisory" issued by the National Association of Realtors warning of the effect of the insulation on the marketability of homes.

Where the ruling may have a more immediate effect on the industry is in how it deals with a spate of liability suits filed in the wake of the commission's ban by homeowners who had already installed the

insulation. A memorandum by one of the industry attorneys summing up the court's decision notes that the ruling of "no evidence of unreasonable cancer risk or of adverse acute effects" should be of substantial help in defence of these actions.

The memorandum also notes that the decision may provide useful ammunition in defending decisions by the Occupational Safety and Health Administration (OSHA) and the Environmental Protection Agency not to pursue further regulatory action on formaldehyde. Both agencies have come under pressure to tighten their regulation of formaldehyde in the light of the cancer studies. OSHA has in fact been sued by the United Auto Workers and several other unions which are demanding an immediate emergency standard to supersede the present 3-p.p.m. workplace limit (based on an eight-hour average; the rule also sets an absolute ceiling of 5 p.p.m.). That case, which was brought last year, has yet to go to trial.

Stephen Budiansky

US accelerator decision

Desertron project gets the vote

Germantown, Maryland

BROOKHAVEN National Laboratory's troubled ISABELLE accelerator project should be scrapped, a high-level federal advisory group said on Monday, and work should begin immediately on a new "Superconducting Super Collider" (SSC). This is a refinement of the "desertron" concept that would accelerate protons up to 20 GeV and, in the words of the group, "assure a forefront US high-energy physics program into the 21st century".

The SSC concept had been strongly endorsed by White House science adviser George Keyworth, who this spring called upon the high-energy physics community to abandon "pet projects" and work together to recapture America's pre-eminence in high-energy physics.

The decision of the High-Energy Physics Advisory Panel's subpanel on new facilities was not reached easily. Although the endorsement of SSC was unanimous, the decision to abandon ISABELLE, now called the Colliding Beam Accelerator (or CBA), was approved by only "a narrow majority". The subpanel's report presents arguments on both sides of the matter; arguments for terminating the project include the "limited opportunity for new and improved physics at CBA" and the "difficulty of attracting the most competent, well trained people to a project that is scientifically not sufficiently exciting".

To some extent, CBA has been overtaken by events. It was designed as a 400 GeV proton-proton collider to search for the W and Z⁰ vector bosons, which have since been detected at the European Organization for Nuclear Research (CERN) in Geneva. And even the arguments in favour of completion of CBA damn it with faint praise, stressing its con-

tribution to the research and development need for SSC and its ability to bridge the gap while SSC is under construction.

In rejecting this view, the majority said that the contributions that CBA could make would be offset by its encumbering rapid construction of SSC.

The SSC concept, as refined at a workshop held in early April at Cornell University, involves colliding 10–20 TeV proton beams, which would probe secondary particles in the mass range of 1–2 TeV. "There is strong agreement that real progress in our understanding of particle physics will come from study of this energy region", subpanel chairman Stan Wojcicki said. The technology necessary — superconducting magnets — is available as a result of the pioneering work at Fermilab in developing the Tevatron.

The subpanel on Monday estimated the cost of construction to be \$2,000 million. In order to achieve operation by the early 1990s, the subpanel recommended an immediate initiation of research and development in fiscal year 1984, with a budget of some \$50 million a year for the next four years. Construction would then take another five years. The cost estimates do not include site costs, which the subpanel assumed would be contributed by the host institution. One design discussed at the Cornell workshop would involve 5 tesla magnets in a 15-km ring.

The subpanel's decision was a sharp blow for Brookhaven, which, after running into "unexpected difficulties" in 1980 with CBA, launched a successful two-year research and development effort to redesign the magnets. Brookhaven is said to be considering a back-up proposal to develop CBA for use as a large heavy-ion accelerator.

Stephen Budiansky

UK energy research

Powers link up with SERC

BRITAIN'S Science and Engineering Research Council (SERC) was cock-a-hoop this week about a three-year agreement with the nationalized energy corporations — the Central Electricity Generating Board (CEGB), the British Gas Corporation and the National Coal Board — to support energy research in universities and polytechnics. The deal between these often warring organizations has taken two years to work out, and even now the bodies will keep each other at arm's length: there will be three separate "joint panels", one between each industry and SERC, to assess projects.

The sum involved is between £600,000 and £750,000, or up to £250,000 per industry per year, half of which will come from the energy corporations and half from SERC. However, at least in the first year, there is to be no new money, just a reallocation of existing support. So the scheme is seen at SERC as only a beginning, a "token of commitment" between the partners. On the council's side, the immediate interest is to maintain the academic quality of corporation-supported research — and only ultimately to raise more money. On the side of the energy corporations, there is interest in influencing, in some degree, the directions of research council policy.

Of the three corporations, CEGB is pushing ahead fastest — and has already placed four grants through the new scheme with the universities of Aston, Reading and Loughborough and with Imperial College, London. One of CEGB's interests is "a long-term dialogue with SERC". At present the board spends some £500,000 each year directly in the universities, of which £50,000 will this year go through the SERC link, to be matched in equal amount by SERC. This is about half the £200,000–£250,000 per industry per year projected by SERC, but CEGB says its total "may rise to that in future years".

SERC's contribution to the scheme will come out of its present energy-related research spending of about £4.5 million a year, which goes to subjects from fusion to materials science and heat pumps. Of this, £1.7 million is channelled through its energy committee, which will have to find the money for the new agreement. The council will respond to the research priorities of the energy corporations, which will have free choice of what projects to support, subject only to the advice of the three joint SERC–industry panels. The boards have a list of priorities which may seem long-term to them but which has a definite short-term flavour by SERC standards. Thus other SERC energy research is likely to be squeezed unless new money is generated.

Robert Walgate

US/Romanian relations

Move to stay in favour

THE threat by the United States to refuse most favoured nation status to Romania when the current agreement expired at the end of June was averted by what amounted to a personal assurance from President Nicolae Ceaucescu to President Reagan: the new law demanding the repayment of higher education costs by those wishing to leave the country would not now be implemented.

A US law from 1974 specifically forbids the granting of most favoured nation status to countries which impose such a restriction on their graduates. The Romanian Government has maintained that the new regulations, which affect mainly Romania's German and Jewish minorities, did not imply ethnic discrimination, but were simply a matter of economics — Romania could not afford to invest in educating experts who would then give their skills to other, richer countries.

Since the fees had to be repaid in hard currency, which Romanian citizens are forbidden to possess, it was clear that their costs would have to be met by friends and relatives abroad. The West German Government, while unwilling to meet the education tax as such, has worked out a scheme of "financial incentives" so that the German emigrants will be exempt — although it refuses to comment on rumours

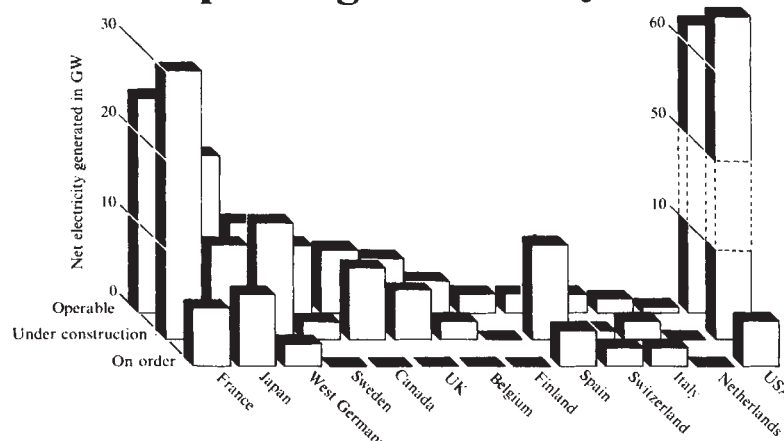
that it is increasing the DM 5,000 at present paid on each German emigrant to around DM 8,000, so that the Romanians will not be out of pocket. Although Israel offered no such incentives, by mid-April a number of Israeli diplomats were confident that the repayment scheme would not be fully implemented.

At the end of May, the Romanian foreign minister Stefan Andrei visited Washington, apparently carrying President Ceaucescu's assurance that the scheme would be dropped. Although Romanian susceptibilities were offended when Mr Edward Derwinski of the State Department publicly called it a "major retreat" by the Romanians, the assurances apparently stood, and the US Government advised Congress to renew most favoured nation status for another term.

Although the US Government pressed for the law to be repealed, it is not clear whether this has been done, or whether it will simply not be endorsed. In the latter case, there would be doubts as to how much reliance can be placed on the Romanian assurances — during 1982, virtually until the law demanding repayment for education was passed on 6 November, President Ceaucescu was assuring Western diplomats that no such law was being prepared.

Vera Rich

The nuclear power game in the year 2000



THE United States dominates the countries of the Organization for Economic Co-operation and Development (OECD) in terms of the net amount of electricity generated by nuclear power, producing more than twice as much nuclear-generated electricity as its closest rival, France. However, despite this massive nuclear production, the United States is well down the nuclear table in percentage terms, producing only 12.6 per cent of its electricity in 1982 through nuclear power. This compares with 40.3 per cent in Finland, 38.7 per cent in France and Sweden and 30.2 per cent in Belgium. The corresponding figure

in the United Kingdom is only 16.4 per cent. The percentage figures for the year 2000 should show the effects of massive nuclear development programmes taking place in France and Spain, with France producing as much as 80 per cent of its electricity through nuclear power and Spain running in second place with 49.4 per cent of its electricity produced through nuclear power. In comparison, the United States is expected to produce 19.8 per cent and Britain 36.6 per cent "nuclear" electricity. Source: *Summary of Nuclear Power and Fuel Cycle Data in OECD Member Countries*, OECD, Paris.

Melanie Kee

University of London

Is big more beautiful?

WHAT could be the biggest upheaval yet in the University of London was signalled last week, when the governing bodies of three constituent colleges of the university, Chelsea College, King's College and Queen Elizabeth College, finally agreed on a formal merger. Even with the reduced numbers of students decreed by the university, the merged college may have close on 6,000 students, excluding some 700 clinical students at King's College Medical School.

With other amalgamations and dismemberments already under way, the upshot of the intended merger will be that the University of London will have contracted from a dozen undergraduate teaching establishments two years ago to five predominant establishments — University College (still the largest with more than 6,000 students and with growing hegemony over Birkbeck College), Imperial College, Queen Mary College (in east London), the amalgam of Bedford College and Royal Holloway College deep in rural Surrey and the now-proposed tripartite merger. In the circumstances, it is not surprising that many senior academics are asking whether the federal university itself will continue to be necessary.

Many of the details of the merger centred on King's College have still to be worked out. The next step will be a binding legal commitment by the three colleges to work

in concert. A unified constitution will be possible, however, only after the passage of a private Act of Parliament and is unlikely to be complete until the beginning of the academic year 1984–85.

One of the most serious academic problems still not solved is the organization of teaching for biology students, expected to number more than 2,000 (including pre-clinical and paramedical students). It is proposed that students should be grouped into three areas, including cell biology and "traditional" biology as well as biomedical sciences, but it is not yet known how student demand will be partitioned.

The cost of implementing the merger is as yet uncertain, but even allowing for the sale of some of the sites at present in use, sums in excess of £12 million have been put forward as the cost of establishing the merged college on a single site.

Meanwhile, Queen Elizabeth College is bitterly resentful of the way in which its budget for the next academic year has been determined, complaining that it was misled by a letter from the university a year ago promising that a then new arrangement for acknowledging the success with which colleges recruited outside support for research should be recognized by a "research factor" phased over three years. This year the expected second tranche has not materialized. □

UK innovation

Sharper image needed

WHAT is to be done about Britain's poor track record in industrial innovation? One answer emerged clearly last week at a conference where industrialists and academics confronted one another: there are no simple answers.

The conference was organized by the Institute of Manpower Studies and the *Times Higher Education Supplement*. Mr Kenneth Durham, chairman of Unilever, said that industry must improve its image with academics in order to encourage more active research collaboration. The government's role should be restricted to facilitating collaboration.

Lord Flowers, newly-appointed chairman of the Committee of Vice-Chancellors and Principals, warned that the autonomy of universities would be threatened if they failed to provide the sort of training that industry needed. He rejected the notion that British university research is too basic: if it were, he said, foreign companies would not find it so interesting. He welcomed the recent joint report of the chairmen of the Advisory Board for the Research Councils and the Advisory Council on Applied Research and Development (see *Nature* 30 June, p.743), which called for a "seed-corn fund" to promote collaboration.

There was general agreement that the monopoly rights on inventions arising from publicly-funded research enjoyed by the British Technology Group had made it more difficult for universities to take their responsibilities seriously. Mr Brian Oakley, head of the Department of Industry's Alvey Directorate, argued that productive academics receive too little support from funding bodies. There is a good case for a national regional research brokerage system to compensate for the inadequacies of the peer review system. But growing nationalism is a potentially serious threat to research collaboration, leading to loss of easy exchange of intellectual property across national boundaries. And, according to Professor John Ashworth of the University of Salford, university hierarchies and attitudes are not geared to industry's need for products delivered on time and to specification. Ashworth revealed that the successes of Salford's "Campus" scheme in fostering productive links with local industry are due in large part to a team of professional managers and public relations staff who chivvy along the academics in the university and attract the interest of industrial companies.

Tim Beardsley

Space Telescope

Planning for refurbishment

Washington

IN the latest of a series of confused signals about the technical status of the Space Telescope, Congress has been warned that the instrument may not be able to perform with its specified accuracy until several years after its launch at the end of 1986. Congressional investigators claim that flaws in the design of the critical fine guidance sensors are so severe that the National Aeronautics and Space Administration (NASA) may launch the telescope with imperfect sensors and replace them with an improved design some time after 1988.

The warning is contained in a report by the investigations staff of the House of Representatives Appropriations Committee. Based on private interviews with unnamed project scientists and NASA officials, the report contradicts many of the relatively optimistic public claims made by NASA. In hearings last month before the House space science subcommittee, NASA administrator James Beggs acknowledged the daunting technical obstacles but maintained that the telescope would conform to all its original design specifications (see *Nature*, 23 June, p.649).

According to the Appropriations Committee report, there is growing doubt within NASA and among the project scientists about the ability of the original fine guidance sensor design to achieve the pointing accuracy required. The report claims that Lockheed warned NASA in 1977 of flaws in the design, but no action was taken to improve it. NASA is now so concerned about the sensors that it has awarded the Applied Physics Laboratory at Johns Hopkins University in Baltimore a \$600,000 contract to design a substitute.

NASA still hopes that the original design, produced by the Perkin-Elmer Corporation in Danbury, Connecticut, can be made to work. The first operational sensor is to be converted into a test model and NASA expects to know by the end of November whether it will indeed be able to "lock-on" to a 14.5 magnitude star as required. If not, the report claims, NASA is ready to consider a fall-back plan under which the telescope would be launched with the imperfect sensors and a substitute design would be inserted into the orbiting telescope by space shuttle astronauts.

The new report does contain a glimmer of good news, however. It confirms claims by NASA that another serious problem — the chafing of the latches on the spacecraft's orbital replacement unit — has been overcome by coating them with tungsten carbide. And the report contains no criticisms of plans to remove dust from the primary mirror by blowing filtered air or dry nitrogen across the surface.

Peter David

Oncogene discovery

Priority by press release

CANCER JAB ON THE WAY. This was the most extreme headline to emerge from the blaze of publicity preceding the simultaneous publication in *Nature* (7 July, p.35) and *Science* (15 July, p.275) last week of independent discoveries of the link between the oncogene of a tumour virus of monkeys and the human gene for a growth-promoting substance produced by blood platelets. Since reasonably accurate and sensible stories in the American press came first, the chauvinism and slavish devotion to an imperfect press release that characterized the British news stories are mainly a reflection of how newspapers operate.

The circumstances were these. Early in May, Dr Michael Waterfield of the Imperial Cancer Research Fund (ICRF) laboratories in London asked Dr Russell Doolittle of the University of California at San Diego for his computerized data base of protein structures. Doolittle willingly sent a tape as it is his policy to make his data base available to any academic scientist.

Waterfield wanted the data base to compare its protein structures with the partial structure of platelet-derived growth factor (PDGF) about to emerge from his laboratory's collaboration with Dr Thomas Deuel's group at Washington University School of Medicine, St Louis, and Dr Bengt Westermark's team at the University of Uppsala in Sweden.

To assist in the search, Waterfield had also asked W.J. Wilbur and David Lipman of the National Institutes of Health (NIH) in Bethesda, Maryland, for their rapid search programs; they also provided, unasked, a data base in their own hands. It was from that data base on 10 May that Waterfield received the astonishing news of the close identity between PDGF and the protein encoded by the simian sarcoma virus cancer gene, the structure of which had been established in Dr Stuart Aaronson's laboratory at NIH. (For those who like coincidences, Wilbur and Lipman's paper was directly followed by Aaronson's in the February issue of *Proceedings of the US National Academy of Sciences U.S.A.*) A search of the Doolittle data base immediately confirmed the discovery.

Seeking biological evidence in support of the sequence data, Waterfield and his collaborators revealed their discovery to a few close colleagues but not to the suppliers of the data bases nor to those from whom they started to request biological materials — to the subsequent annoyance of many. Plans changed, however, with the publication in the 27 May issue of *Science* of a partial structure of PDGF by Michael Hunkapillar of the California Institute of Technology and Harry Antoniades of the Harvard School of Public Health. (To confuse matters, *Science* is published a week ahead of its cover date.)

To the relief of the Waterfield team,

Hunkapillar and Antoniades had not spotted the relationship between PDGF and the cancer gene, stating that "a limited search . . . has not revealed any significant homology . . .". Realizing, however, that it could not be long before somebody spotted the relationship from the published data, Waterfield telephoned *Nature* in the middle of the week of 22 May to ask if his paper could be published quickly, once he had produced a final draft after returning from a holiday on Rhodes.

Events then moved quickly. On 28 May, Russell Doolittle read the Hunkapillar and Antoniades paper, fed their PDGF struc-

ture into his data base and reached the same result as Waterfield. He then alerted Hunkapillar and Aaronson and word spread from the former to his colleague Leroy Hood, who made it public during a meeting of the American Society of Biochemistry in San Francisco in the week of 5 June. As it happens, Waterfield's collaborator, Deuel, was at Hood's talk, and Waterfield's worship of Helios on Rhodes was soon interrupted by the news; the paper by Waterfield *et al.* was submitted to *Nature* on 14 June, a couple of days before Waterfield's return to ICRF. Meanwhile, Doolittle *et al.* had already sent their paper to *Science* on 3 June.

News of the discovery first reached a wider public through the 18 June issue of *Science News*, a reporter from which had heard Hood's talk. By the end of June, the story had spread through the *Los Angeles Times* to the *Washington Post* and the *New York Times*. Although some of the writers had picked up rumour of Waterfield's work, it was Doolittle's that gained prominence. By 1 July, the story was in the *International Herald Tribune* and the Imperial Cancer Research Fund feared it would gain no credit for Waterfield's prior discovery. With *Nature*'s approval, the fund issued a press release on that day, a week before publication.

The press release was not cautious. It did not mention the fact that the only strong link of the discovery to cancer was in the case of tumours of monkeys caused by simian sarcoma virus. It claimed categorically that PDGF "produced by the oncogene in excessive amounts . . . leads to uncontrolled growth in . . . bones and other connective tissue" and that drugs can now be devised to block the effects of PDGF and "perhaps stop the cancerous growth".

Not surprisingly, the story was in every television news bulletin that night and prominently dealt with in every British newspaper the next morning. Almost without exception, the reports closely followed the press release. None made clear the viral context of the discovery or the degree of extrapolation from that to human tumours. Only one mentioned Doolittle *et al.* And all told of possible drugs, mostly with due regard for the speculative nature of that possibility.

Not so the *News of the World*, a Sunday paper that likes to titillate prurient interests of its readers from a platform of moral indignation. "A superjab that will cure certain types of cancer will be available in Britain within a year", it began, quoting Waterfield as saying "I would honestly hope that within the year. . . we will find a drug to halt some kinds of cancer". Asked last week if that was an accurate quotation, Waterfield said he could not be certain exactly what he did say in response to the leading questions of a persistent reporter — one who had begun by asking if he was in cancer research because a relative was suffering from the disease. **Peter Newmark**

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

From last week's *News of the World*.

European Community

Stronger research committee

Brussels

THERE is a newcomer to the European Commission's arsenal of scientific think tanks. The new committee is called Codest, the Committee for European Development of Science and Technology, and its aim is to promote the neglected "European dimension" of basic scientific research.

Chaired by Dr Umberto Colombo, president of the Italian board for nuclear and alternative energies, Codest's bureau is co-managed by Ilya Prigogine, a Nobel prize-winner for chemistry, and by Hubert Curien, president of the European Space Agency and chairman of the European Science Foundation. Sir David Phillips, who heads the British Advisory Board for Research Councils, and Professor Benno Hess of West Germany's Max Planck Gesellschaft are bureau members — eighteen eminent scientists from all member states complete the committee.

Scepticism usually greets the creation of yet another consultative committee, but after Codest's first bureau meeting last week, research commissioner Etienne Davignon was quick to point out the essential differences between this latest creation and other groups. "Codest members don't represent their country or their government. They're scientists and have an aversion to bureaucracy", Davignon said. And in a clear allusion to the poor performance of Crest, a committee of scientific delegates of all member governments designed to advise the European Commission on matters involving Community research, Davignon added: "In many of the so-called expert committees of the European Community, it is not always obvious that so-called experts have real expertise".

Codest is part of a programme aimed at stimulating Community-wide research and development and in that role was allocated a two-year budget of 7 million ECU (1 ECU = £0.58) by European Community research ministers last month. Codest runs parallel to and complements the programme for forecasting and assessment in the field of science and technology (FAST).

The new committee has already launched an appeal to all European researchers, urging them to submit ideas for research programmes in the fields of pharmacology, health and drugs, solid state physics and new materials, optics, combustion, photometrics and photo-acoustics, and climatology. A first examination of some of the projects is planned in October, when the committee has scheduled its first plenary meeting. "We don't want to limit the scope of Codest to these seven areas, but it's in these fields that the gap between basic research and research into industrial applicability is widest", Dr Colombo explained.

A major task of Codest, says Colombo,

will be to promote mobility of European researchers. "Europe accounts for 20 per cent of the world's research, employing more than a million workers of whom 150,000 are qualified researchers, but

there's very little coordination at European level", Colombo said. "Yet there have been successes: the European nuclear fusion programme ranks among the most ambitious in the world and the Ariane space launcher is now proving its mettle. But European researchers don't identify themselves with Europe. There lies a major task." **Geert Linnebank**

Biotechnology

Still on the up and up?

WHILE the *Nature* biotechnology index continues to prosper (see below), companies in the United States and elsewhere appear to have entered a new phase of wheeling and dealing with each other. In Britain, for example, Celltech (backed to the tune of 40 per cent by the British Government) modestly increased its share capital and, perhaps more significantly, increased the diversity of its shareholders.

At the same time, Celltech has signed an agreement with Boots Ltd, best known for its chain of retail drug stores selling hot-water bottles as well as prescription drugs, to market to hospitals and physicians a series of diagnostic products based on monoclonal antibodies.

What seems to be happening is that biotechnology companies are being persuaded by events, and by the comparative ease with which variants on one kind of product can be readied for the market, that the involvement of companies with experience of selling products would be beneficial. No doubt the run-down of initial investment capital has argued in the same direction.

Meanwhile, optimism about the commercial prospects of important biotechnology companies appears to be reviving after two years during which financial investors appear to have been persuaded that

the field is overcrowded. While it is unlikely in the future to be more difficult than say two years ago for new biotechnology companies to recruit their initial capital requirements, stockbrokers seem recently to have been drawing the attention of their clients to the attractions of companies with a solid foundation in pharmaceuticals but with a substantial interest in biotechnology as well.

Novo Industries, the Danish company which is the chief supplier of human insulin in Western Europe and Canada, and whose sales and profits have increased by more than a fifth in each of the past five years, is one of these.

Even so, optimism seems not to be universal. Even though Amersham International, the supplier of radiochemicals whose shares were disposed of a year ago by the British Government, recorded a substantial increase of sales profits during its first year, the London Stock Exchange appears to have been expecting even more with the result that the price of its shares drifted modestly downwards. Part of the underlying calculation appears to have been that the increase in the scale of biotechnology research should have brought a corresponding increase in Amersham's activities. □

Nature index of biotechnology stocks

12-Month high	12-Month low	Company	Close previous month	Close 27 May	Change
90½	45¼	A.B. Fortia (Sweden)	82	77	-5
23¼	15¾	Biogen (Switzerland)	16¾	15¾	-1
6¼	3	Bio-Logicals (Canada)	4¾	5½	+¾
14	7¼	Bio-Response (USA)	10¾	14	+3¾
19	11¾	Cetus (USA)	16½	17¾	+1½
15¾	8¾	Collaborative Research (USA)	9½	15¾	+5¾
39¾	15	Damon (USA)	37¾	32¼	-5½
41	24½	Enzo-Biochem (USA)	39¾	30½	-9¼
18¾	10¾	Flow General (USA)	13¼	15	+1¾
46½	26¾	Genentech (USA)	42½	41¼	-¾
17¾	8¾	Genetic Systems (USA)	12¾	16¾	+4½
20¼	12¾	Genex (USA)	18½	18½	0
31	21¼	Hybritech (USA)	26¾	27¼	+½
22¼	13¾	Molecular Genetics (USA)	22	21½	-½
23¼	16	Monoclonal Antibodies (USA)	19¼	18	-1¼
65¾	42	Novo Industri A/S (Denmark)	60¾	63¾	+3¾

Closing prices are for the last Friday of the month. For over-the-counter stocks, bid price is quoted; for stocks on the American and New York exchanges, the transaction price. The index stood at 233 on 24 June, compared with 223 a month earlier. Data from E.F. Hutton, Inc.

Marine animal killing

SIR — D.E. Sergeant is correct in claiming that work on humane killing of seals and whales is intermittent and under-financed (*Nature* 17 February, p.560). Sadly, this is likely to remain so while those engaged in such activities place profit margins above all other considerations.

Dr Sergeant thinks we are "passing through a period when whaling and sealing are socially unacceptable". I am not sure that is so, but I think we are passing into a period in which it is not acceptable that industries exploit living marine resources except insofar as it can be ensured, from scientific assessments, that the level of exploitation is sustainable.

The reason why those with no particular axe to grind are so upset with the Canadian Government over the "hunt" for young harp and hooded seals is that the administration of Fisheries and Oceans Canada has been so adamant in refusing to acknowledge what most scientists who have studied the data believe — that there is enormous uncertainty about the numbers of animals that remain, and about whether they have increased in numbers in recent years or declined under the quota system. In this situation any administration truly concerned with the continuing productivity of a slowly renewable resource would cut back quotas for "safety".

Dr Sergeant says that "when hunting pressure is relaxed sea mammal populations rise to near primaevial levels", so long as "we maintain their food base, and the water quality of the near-seas". But there is little empirical evidence for that assertion. The idea of "full recovery" comes essentially from running backwards population models developed from studies of depletion of whaling or sealing. Most of those simplistic models have recently been called into question, and they certainly are not reliable for predicting recovery.

The only way we could conceivably be sure of natural population increases is by direct monitoring. It is therefore deeply to be regretted that in recent years the Canadian Government has not funded any direct census of harp and hooded seals.

Dr Sergeant's assertion about recovery to primaevial levels begs the question about the food base of the seals. In the mid-1960s the fishery for capelin, on which the seals are said largely to depend, collapsed due to over-fishing. In the same period Dr D. Lavigne, working at Guelph, Ontario, showed that the "condition" of the seals had deteriorated. It is unlikely that in those conditions the survival rates of pups would have been unchanged. Since then there has been some recovery of the capelin, but the consequences of these changes for the seals have not yet been worked out.

SIDNEY J. HOLT

*Symposium on Marine Mammals
of the Indian Ocean,
Colombo, Sri Lanka*

No to protection for authors

SIR — With regard to your response to Raymond Bradley in the 26 May issue of *Nature* (p.278) to the effect that you obtain copyright to journal articles "so that the authors' work can then be protected" really does not make any sense as far as the authors are concerned. As one of your authors, I can say that I have no wish to be so protected. When an author publishes an article in a scientific journal, he wishes to be referenced and discussed, not protected. I believe that the most elementary poll of your authors will show that they too do not wish to be protected by copyright, and further that their interest would be best served if their articles were not copyrighted at all.

In your reply to Professor Bradley you state that the administration of permissions for copying figures or tables from articles is burdensome for busy journals such as *Nature*. Why not then, just as an experiment, try for, say, 1 year the policy suggested by Professor Bradley of automatically granting permission for copying figures and tables from your journal provided proper credit is given? If something bad starts to happen, you can revert to your old policy.

A.J. DESSLER

*Marshall Space Flight Center,
Alabama, USA*

Scripps' foundation

SIR — In your issue of 12 May (p.126), where you memorialize Adriano Buzzati Traverso, you describe him as a co-founder of the Scripps Institution of Oceanography.

That he was not. The institution was formally founded in 1903 and had already won an international reputation as a centre for marine science when he joined its faculty in 1952.

I suspect that what was referred to was his association with Roger Revelle when the latter started what was to become the University of California at San Diego.

WILLIAM A. NIERENBERG

*University of California, San Diego,
La Jolla, California, USA*

Energy shortage?

SIR — In his report on French budgets (*Nature* 19 May, p.193), Robert Walgate abbreviates the CNRS special programmes (*actions thematiques programmées*) to ATP. Surely, *real* ATP is more essential for the French molecular biology programme than this pseudo ATP!

J.J.A. HEFFRON

*Department of Biochemistry,
University College, Cork, Ireland*

Stereo viewing

SIR — This letter is prompted by N. Michael Green's letter on stereo viewing (*Nature* 27 January, p.279). I, and many of my friends and colleagues, have found that stereo pictures can be viewed easily without a stereo viewer or any other such device.

The trick is to focus behind the plane of the pictures so that each of the pictures is viewed as double. The focus then needs to be adjusted so that the separation between the doubles becomes just correct to superimpose the right-hand image of the left-hand picture and the left-hand image of the right-hand picture. The line joining the pictures should be parallel to the line joining the two eyes. This can be achieved by rotating the page slightly if necessary.

The book *Physics for Entertainment*, written by Ya Perelman (Mir Publishers) gives a "graded course" in stereo-picture viewing through a series of stereo pairs of increasing detail.

D.G. BANHATTI

*Radio Astronomy Centre,
Ootacamund,
Tamil Nadu, India*

Sir Cyril Burt's "missing ladies"

SIR — Alerted by W.F. Bynum's positive review¹ and later the half-page advertisement², I have now obtained Nancy Stepan's interesting book *The Idea of Race in Science: Great Britain 1800-1960*.

On her pages 187 and 224, it is disconcerting indeed to find the first name Jill given to the Ms Conway whom Oliver Gillie has documented in *Science*³ to be one of Sir Cyril Burt's "Missing Ladies" and about whom Gillie has reported "Conway's case is . . . curious. No one . . . knows anything . . . about her . . . Jensen has given her the name Jane . . . but I can find no documentary evidence for this name". Furthermore, neither the source^{4,5} cited by Stepan for this tale of charlatanry⁶ uses any name other than the J. Conway which appears only in that form throughout the Burt literature.

Certainly it shakes our confidence when author and reviewer (who is cited in the Introduction by the author in support of one of her three basic assumptions) pass over in silence such a bizarre development in a very notorious case.

JERRY HIRSCH

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2. *Nature* 20 January 1983, Reader Service Card No.45.

3. Gillie, O. *Science* 204, 1036 (1979).

4. Hearnshaw, L.S. *Cyril Burt, Psychologist* (Hodder & Stoughton, London, 1979).

5. Kamin, L.J. *The Science and Politics of IQ* (Lawrence Erlbaum Associates, Potomac, Maryland, 1974).

6. Hirsch, J. *SAGE Race Relations Abstracts* 6 (2), 1 (1981).

The function of dream sleep

Francis Crick* & Graeme Mitchison*

We propose that the function of dream sleep (more properly rapid-eye movement or REM sleep) is to remove certain undesirable modes of interaction in networks of cells in the cerebral cortex. We postulate that this is done in REM sleep by a reverse learning mechanism (see also p.158), so that the trace in the brain of the unconscious dream is weakened, rather than strengthened, by the dream.

MANKIND has always been fascinated by dreams. As might be expected, there have been many attempts to assign a purpose or significance to them. Although we dream for one or two hours every night, we do not remember most of our dreams. Earlier thinkers, such as Freud, did not know this. Modern theories (not reviewed here in detail) have usually proposed that sleep and dreams save energy or have various restorative functions, either to replenish the brain biochemically in some way, or to reclassify or reorder the information stored in it.

Sleep is of several kinds. Dream sleep, or rapid eye movement (REM) sleep, is predominantly found in viviparous mammals and birds. It seems to be associated with homeothermy (a constant internal temperature) and the possession of an appreciable neocortex or its equivalent. It is not unimportant because of the appreciable amount of time we spend in this peculiar state.

We propose here a new explanation for the function of REM sleep. The basis of our theory is the assumption that in viviparous mammals the cortical system (the cerebral cortex and some of its associated subcortical structures) can be regarded as a network of interconnected cells which can support a great variety of modes of mutual excitation. Such a system is likely to be subject to unwanted or 'parasitic' modes of behaviour, which arise as it is disturbed either by the growth of the brain or by the modifications produced by experience. We propose that such modes are detected and suppressed by a special mechanism which operates during REM sleep and has the character of an active process which is, loosely speaking, the opposite of learning. We call this 'reverse learning' or 'unlearning'. This mechanism, which is not the same as normal forgetting,

is explained in more detail below. Without it we believe that the mammalian cortex could not perform so well.

We first describe our ideas about the cortex followed by a brief account of neural networks. Next we outline what is known about REM sleep. (For general accounts, see refs 1,2.) We then describe our postulated mechanism and how it might be tested. Finally we discuss various implications of our ideas.

The cortex

The cortex consists of two separate sheets of neural tissue, one on each side of the head. The neocortex, which has a characteristic layered structure, is found only in mammals (see ref. 3 for recent survey), although a somewhat analogous structure, the wulst, is found in birds. If allowance is made for body weight, it is larger in primates than in most other mammals and larger in man than in other primates. It makes up a substantial fraction of the human brain.

Different areas of the cortex perform different functions, some being mainly associated with vision, touch and so on, while others appear to process more complex information not associated with a single sensory mode. The exact function of the neocortex is unknown but it appears to be closely associated with higher mental activities. It seems likely that it has evolved to perform in a rather special way.

In examining the neuroanatomy of the neocortex one is struck by the very large number of axon collaterals (this is not true, for instance, of the thalamus). In any area of the cortex the great majority of synapses come from axons originating locally and running within it. There is also evidence that the majority of the synapses in the cortex are excitatory in their action. This suggests a capacity for self-excitatory modes of behaviour in the cortex. And indeed, in various conditions, such as epilepsy, migraine and certain kinds of drug-induced hallucination⁴, parts of the cortex appear

to go into large-amplitude instabilities⁵.

Neuronal networks

Now, if one asks what functions such richly interconnected assemblies of cells could serve, one attractive possibility is that they could store associations⁶⁻⁸. To see this, suppose an 'event' is represented by the activity of a subset of cells in a cell assembly. If all the cells involved in that event form mutual synapses, then when part of that event is encountered again these synapses can cause the regeneration of the activity in the entire subset.

Much exploratory theoretical work has been done on such networks of cells (for an introduction see refs 6-8). In these models, information is stored in the strengths of the many synapses and sometimes in the firing thresholds of cells as well. Although the exact behaviour naturally depends on the details of the particular model, certain general properties can emerge even from relatively simple models. The associations which are stored are not assigned specific locations for each item, as in a digital computer. Instead the information is: (1) Distributed: this implies that a particular piece of information is distributed over very many synapses. (2) Robust: this implies that the information will not be totally lost if a few synapses are added or removed. (3) Superimposed: this implies that one synapse is involved in storing several distinct pieces of information.

A properly designed net can be trained (meaning that the strengths of the synapses can be adjusted) so that given an input (a pattern of axonal firings) it can produce the appropriate output (another pattern of axonal firings). It is found that certain general properties will often emerge. (1) Completion: given only part of the input (as a clue) it can produce fairly exactly the whole of the appropriate output (examples are given in ref. 7). In computer jargon, the memory is 'content addressable'. (2) Classification: given an input which is related to several of its associations, it may

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produce an output which combines many of the common features of its normal outputs.

A major difficulty with all nets of this general type is that they become overloaded if an attempt is made to store simultaneously too many different patterns or associations of patterns, or if the stored patterns have too large an overlap. This is because of the superimposed nature of the storage. How the net will behave when overloaded depends on the exact structure of the net, but certain patterns of behaviour are likely to emerge: (1) The net may produce many far-fetched or bizarre associations ('fantasy'). (2) The net may tend to produce the same state, or one of a small set of states, whatever the input ('obsession'). (3) Certain kinds of nets, particularly those which feed back on themselves, may respond to inappropriate input signals which would normally evoke no response from the net ('hallucination').

It is against this background of rather tentative and idealized theory that our proposals must be judged.

If the cortex were hard-wired during embryogenesis to an exactly predetermined pattern of synaptic connections, the burden of eliminating parasitic modes in cortical nets would have to be undertaken by the genes alone. Although there is considerable evidence for specificity in the cortical wiring, it is likely that many of the details of the synaptic connections — their exact locations and their strength — are made in a semirandom manner and refined by experience. This is almost a necessity in an organism which is capable of learning very large amounts of novel information. Thus it seems likely that both during cortical growth (when we may say that certain broadly predetermined 'associations' are laid down), and also in facing the experiences of adult life, such parasitic modes will be unavoidably generated.

How would one attempt to eliminate these modes? We suggest the following. The major inputs and outputs of the system should be turned off, so that the system is largely isolated. It should then be given successive 'random' activations, from internal sources, so that any incipient parasitic modes would be excited, especially if the general balance of excitation to inhibition had been temporarily tilted towards excitation. Some mechanism is then needed to make changes so that these potentially parasitic modes are damped down. Such a rough outline description immediately reminds one of REM sleep and the hallucinoid dreams associated with it.

REM sleep

It was discovered in the 1950s that in mammals there are two main types of sleep. Periods of REM sleep (also called D sleep or paradoxical sleep) alternate with periods of non-REM sleep (also called S sleep, slow-wave sleep, or orthodox sleep) of which four stages of increasing depth of sleep are usually distinguished. During

REM periods may of the muscles of the sleeping animal, especially its head and neck muscles, are more relaxed than in non-REM sleep. Its cortex, as judged by the electroencephalogram (EEG) and by the rapid movement of the eyes beneath closed lids, appears to be very active and in a state similar to the waking state. On the other hand, the monoamine neurones in the brain stem, especially those in the locus coeruleus, raphe and peribrachial nuclei, reduce their firing rates in REM sleep to only a few per cent of the corresponding rate in the waking state⁹.

Another major difference between REM and non-REM sleep lies in the dreams associated with them. For most people the few dreams found in non-REM sleep tend to have a rather thought-like character. During REM sleep, on the other hand, dreams occur more frequently and usually have a perceptual vividness and the illogical episodic character with which we are all familiar. A human adult usually spends a total of 1½ to 2 hours each night in REM sleep, spread over several periods. The evidence suggests that most of the dreams during these REM periods do not reach normal consciousness, dreams being remembered only if the sleeper awakes while dreaming. Even then the memory of a dream is usually very transient, fading quickly if no effort is made to remember it by rehearsing its content.

A most remarkable finding is that newborn humans may have as much as 8 hours of REM sleep per day¹⁰. There is also evidence to suggest that in the womb, especially in the third trimester, REM sleep occurs even more frequently. This large amount of REM sleep before and after birth is also found in other mammals.

All viviparous mammals examined, including primitive marsupials such as the opossum, show periods of REM sleep^{11,12}. Even an animal like the mole, which can hardly move its eyes, shows the characteristic EEG of REM sleep. Birds have REM sleep, although often only a very small amount of it, occupying perhaps 5% of their sleep¹³. There are no very convincing reports of REM sleep (as judged by the EEG) in reptiles, amphibians or fish.

If an animal is deprived of REM sleep for one or more nights (but allowed non-REM sleep) then it will usually have more REM sleep in subsequent nights^{14,15}.

All this evidence suggests that REM sleep has an important function, at least for mammals. Since the majority of dreams are not remembered, that function is more likely to be associated with the unconscious dreaming process — that is, with REM sleep without awakening — rather than with the few dreams which are recalled.

It has been shown that during REM sleep the forebrain is periodically and widely stimulated by the brain stem. This activity in the brain stem can happen even in the absence of the cortex. Hobson and McCarley¹⁶, following the pioneer work of

Jouvet¹⁷, have postulated a 'dream state generator' which lies mainly in the pontine reticular formation (the question of which exact cell groups are involved is controversial). It produces the so-called PGO waves. They propose that the activity of such cells is the cause of both rapid eye movements and the periodic intrusion of new subject matter into hallucinoid dreams. Our proposals are based on this idea.

In summary, the evidence suggests that in REM sleep the brain is isolated from its normal input and output channels and that it is very active, this activity being promoted by rather nonspecific signals from the brain stem and reflected in the unconscious equivalent of dreaming, which only reaches normal consciousness if the sleeper awakes.

The postulated mechanism

We need a mechanism which will tune the cortical system, in the sense of removing parasitic modes which arise after the system has been disturbed either by growth of the brain (when new connections are constantly being made) or by the modifications produced by experience. The mechanism we propose is based on the more or less random stimulation of the forebrain by the brain stem that will tend to excite the inappropriate modes of brain activity referred to earlier, and especially those which are too prone to be set off by random noise rather than by highly structured specific signals. We further postulate a reverse learning mechanism which will modify the cortex (for example, by altering the strengths of individual synapses) in such a way that this particular activity is less likely in the future. For example, if a synapse needs to be strengthened in order to remember something, then in reverse learning it would be weakened. Put more loosely, we suggest that in REM sleep we unlearn our unconscious dreams. "We dream in order to forget."

After this paper had been initially submitted for publication, we learnt from Dr John Hopfield that he and his colleagues had independently arrived at the idea of reverse learning, though not in connection with dreams. In a parallel communication¹⁸ they have shown that the behaviour of their very idealized neural net is indeed improved by reverse learning. That is, it equalizes the accessibility of stored memories and suppresses most of the spurious ones. We have since repeated their simulations and confirmed their general conclusions. It remains to be seen how well reverse learning acts on other more realistic neural nets. We have revised our paper in the light of their results.

Note that the *amount* of reverse learning per step in these simulations was very small (only about 1% of the amount needed for complete learning), although several hundred such steps were used. This alerts us to the possibility that the changes produced in REM sleep may individually be very small but cumulative over many PGO spikes and

many nights' sleep.

The objection might be raised that some experiments have shown that REM may appear to help the retention of memory, whereas the process of reverse learning would tend to make the memory fade. The results of Hopfield *et al.*¹⁸ show that this need not be the case. After reverse learning the recall of their net was less confused and more uniform.

If there is indeed a mechanism for reverse learning, many questions arise about its character. Does it act via the same mechanism as normal learning (whatever that is) or is a special, quite separate, mechanism involved? Is the mechanism associated with one particular system in the brain stem? Another possibility is that a small amount of reverse learning is always present but is normally overwhelmed by the positive plasticity produced by one or more of the diffuse systems from the brain stem. When their activity is greatly reduced, as it is in REM sleep⁹, the residual reverse learning can then exert its effect unopposed, at least on recently modified synapses.

In its simplest form our theory postulates that there is no intelligent supervisor inside the brain which decides in detail which potential neural activities should be left untouched and which should be damped down. This choice is made solely by the response of the forebrain to the relatively nonspecific signals from the brain stem. In very general terms, the brain stem gives the forebrain a varied pattern of bangs (the PGO waves). Any resulting activity is then modified so that it is less likely to occur in the future.

It would of course be possible to postulate a more complex mechanism. For example, in REM sleep, especially in early development, there could be innate testing programmes, together with a 'supervisor' to decide what to store and what to erase, depending on the result of the tests. Various workers have made proposals along these lines¹⁹⁻²⁴. As far as we know, nobody has previously suggested that the testing procedure involves the removal of potentially parasitic modes.

It has been customary to believe that during an unconscious dream the content of the dream is stored in some form of very short-term 'memory' but that the mechanism for transferring it into longer term memory is inoperative. We normally become conscious of our dreams only if we wake up while dreaming is in progress. If we then pay attention to our dream, some of its content can be maintained in very short-term memory and may eventually be transferred to longer-term memory as the transfer mechanism becomes activated. Otherwise our dream fades. Thus we can speak of forgetting our dreams, meaning that we know that we had a dream, but are somewhat uncertain of its content.

This forgetting of a dream, which has often been remarked on, does not necessarily involve our postulated reverse

learning process. The latter is a positive mechanism which does not merely fail to alter synaptic strengths (or other long-lasting brain parameters) but changes them so that the dream is not just forgotten but actively 'unlearned'. The result is that the dream (or some of the elements of it) is less likely to recur in the future.

The terms 'reverse learning' or 'unlearning' are not ideal because they rather imply that one has to learn something first in order to unlearn it. What does a fetus 'learn' that has to be unlearned? Our answer is that, during development, the semirandom process of making synaptic connections is likely to produce parasitic modes. It is these which must be 'unlearned' in order to obtain a well-behaved system.

We need some explanation for recurrent dreams. We propose the *ad hoc* hypothesis that a recurrent dream is one which, for one reason or another, tends to wake up the sleeper, perhaps because of the anxiety often associated with them. This will have the effect that the learning process changes sign, passing from reverse learning to positive learning, so that the underlying spurious associations remain, and so a similar dream is likely to occur on some later occasion. This mechanism does postulate a supervisor of a kind but its sole function is to decide whether the sleeper should wake up or not. Thus for a dream to become recurrent it must have two properties. It must be related to a potentially parasitic mode and it must wake up the dreamer in such a way that he remembers it rather vividly.

Our theory, in its present state, says nothing about the function of non-REM sleep. These stages of sleep usually have less of the hallucinoid type of dream which we associate with our reverse learning mechanism. Non-REM sleep is likely to have the restorative function often postulated for it but it may also have some informational function. For example, it might be used for the process of 'consolidating' memory in some way. It is worth noting that the first REM period of the night is normally preceded by a substantial period of non-REM sleep.

Testing the theory

As far as we can tell, our theory is broadly compatible with a large amount of experimental data. Starting from a plausible hypothesis about cortical function, it explains in an effortless way both the need for REM sleep in adult life and the large amount of it during the development of the brain. We believe no previous theory explains this distribution of REM sleep in such a simple manner. Any purely psychological theory (such as Freud's) is hard-pressed to explain the large amount of REM sleep in the womb, and any purely developmental theory must account for the quite appreciable amount of REM sleep in adult life. Our theory accounts for both. It is also compatible with the hallucinoid

nature of REM dreams.

The effects of REM sleep deprivation are harder to explain. It is well established that REM deprivation often produces a rebound — more REM sleep than usual occurs when the subject is eventually allowed to sleep without interruption. We would have expected that REM deprivation, if severe enough, might cause hallucinations — that is, structured visual and auditory responses to 'noise' — and perhaps delusions and obsessions. There is a little evidence for this²⁵, but usually the effects are either small or absent²⁶. This is partly because it is extremely difficult to produce long periods of complete REM deprivation in humans by selective arousal. After a week or two it becomes almost impossible to awaken them promptly at every onset of REM sleep, so that prolonged experiments have not been done. One cannot help but wonder whether similar experiments on food deprivation might lead to the conclusion (if unsupported by other evidence) that food also had no essential function. However, REM deprivation in animals does appear to lower the threshold for cortical instability produced by electroconvulsive shock²⁷⁻³⁰, which is what we might expect. REM deprivation in humans sometimes produces irritability and an inability to concentrate. One might suggest that these are the effects of the attention mechanism being forced to subdue sub-threshold parasitic modes which would otherwise break into consciousness. REM deprivation can also allow feelings and wishes to appear which had previously been kept out of consciousness³¹, or, in certain subjects, can show changes towards increased internal fantasy during waking³².

A further difficulty is that some drugs, such as certain monoamine oxidase inhibitors, appear to prevent REM sleep entirely³³ without producing very obvious psychological deficits. This is a difficulty for any theory which assumes REM sleep is important and runs in the face of all the other evidence about it. We can only plead that such drugs may have complicated side effects which make the observations misleading.

A direct test of our postulated reverse learning mechanism seems extremely difficult. It would be necessary to show that our unconscious dreams (dreams we do not remember — a new word for this is really needed, we suggest 'remination') reduce the probability of such thoughts occurring in the future. This is far beyond the methods we have available today. It would be interesting to know if the threshold for hallucination, induced by drugs or other means, is lowered as a result of REM deprivation. Another approach would be to look for the structural and chemical correlates of the postulated reverse learning mechanism, but exactly how to do this is at the moment unclear. Without further evidence of this kind our theory must be regarded as speculative.

It is clear that useful insights can come

from neural modelling. This approach has its limitations, since it is difficult to produce realistic models and even more difficult to simulate them effectively, especially if the hypothetical neural nets approach a realistic size, when the computational time becomes prohibitively long. However, such theoretical studies should at least reveal some of the types of networks which would benefit from our proposed mechanism. They might also help to give more life to our otherwise rather vague characterization of the cortical system.

Another approach would be to undertake comparative studies. There is one mammal which, although possessing a well developed neocortex³⁴, appears not to show any signs of REM sleep (at least in young adults), even though it exhibits normal non-REM sleep³⁵. This is the Echidna *Tachyglossus aculeatus* (the spiny anteater) found in Australia. The Echidnas and the duck-billed platypus are primitive egg-laying mammals (monotremes).

Griffiths³⁴ has written that "... the gyrencephalic cerebrums of the Tachyglossidae have been and are a source of wonder to neurobiologists". He quotes Elliott Smith³⁶ who in 1902 wrote "The most obtrusive feature of this brain is the relatively enormous development of the cerebral hemispheres ... The meaning of this large neopallium is quite incomprehensible ...". Griffiths adds: "Determinants of modern neurophysiology also fail to explain how echidnas come by this cortex". We suggest that *Tachyglossus* needs such a large cortex because it cannot tune it up by the process of reverse learning. Experience with idealized neural nets shows that one can usually avoid overloading a net, and thus the confusion such an overloading creates, by making the net bigger.

Tachyglossus can be studied in captivity. It might be rewarding to examine in more detail its behaviour, neurophysiology and neuroanatomy compared to a primitive placental mammal, such as a hedgehog, which does show REM sleep. If REM sleep serves an important function, this should be reflected in some way in its absence in the spiny anteater.

Possible implications

If it turns out that our ideas are broadly correct, they could help us to understand the evolution of the neocortex which is so typical of mammals. It seems likely that in order for a highly tuned system to perform efficiently at least two requirements are

necessary: a fairly constant internal temperature, so that its function is not disturbed by temperature fluctuations, and in addition a cleaning-up mechanism, to remove potentially parasitic modes. In short, without REM dreams evolution could not have produced the highly refined neocortex we have today.

If the reverse learning mechanism we have postulated exists, one might wonder what effects its failure might have. A complete failure might lead to such grave disturbances — a state of almost perpetual obsession or spurious, hallucinatory associations — that it would probably be severely selected against. A partial failure should produce unwanted responses to random noise, perhaps as hallucinations, delusions, and obsessions, and produce a state not unlike some schizophrenias.

It has been postulated before that there might be a relation between REM sleep and schizophrenia, but studies have shown that there is little or no connection between the outward signs of REM sleep and schizophrenia³⁷. However, a partial failure of the reverse learning mechanism would not necessarily alter the amount of REM sleep, since the control mechanisms for the occurrence of REM sleep might be somewhat distinct from the reverse learning process itself. Thus the possibility that some forms of schizophrenia might be

caused by a defect in the reverse learning process should not be overlooked.

In this model, attempting to remember one's dreams should perhaps not be encouraged, because such remembering may help to retain patterns of thought which are better forgotten. These are the very patterns the organism was attempting to damp down.

Finally we should remark that even if it turns out that our ideas are wrong and that nature does not employ the reverse learning mechanism we have postulated, the process may well be useful for artificial intelligence machines of the future, especially those having extensive parallel processing, a learning mechanism and a certain amount of randomness in their construction.

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Chaos theory infects civil engineers

Recent interest in the theory of chaos has been prompted largely by the wish to understand the inexplicable. But there may be serious practical consequences for engineers.

GIVEN the degree to which most people's daily lives depend on engineered structures, it is a relief that civil engineers have now been infected by the pre-occupation with chaos that seems for the past three years to have gripped mathematicians, physicists and even biologists. Chaotic systems are those in which the behaviour of a strictly well-determined system, mechanical or otherwise, cannot be simply inferred from a knowledge of its behaviour in a few selected circumstances (see *Nature* 303, 15; 1983). The simplest image is movement of a metal sphere from the top to the bottom of a pinball machine — every interaction of the sphere with an obstacle is governed by newtonian mechanics, but the outcome (the position of the ball on the bottom of the machine) is so sensitively dependent on the initial conditions that it seems to be randomly determined. If engineered structures were commonly plagued by such behaviour, we should all sleep less easily at nights.

In the most recent issue of *Proceedings of the Royal Society* (A307, 407–427; 1983), Dr J.M.T. Thompson of University College London points to a class of engineered structures whose behaviour may indeed be chaotic. Thompson's calculations stem from another fashion among British researchers — the design of marine structures such as those used for extracting petroleum from the North Sea. As a potential source of chaos, he offers the oscillation of an oil-production facility pinned to the ocean floor and supported more or less vertically by buoyancy. Unencumbered, such a structure is simply an easily calculable inverted pendulum, but if an oil tanker should be attached to it by mooring lines, the problem becomes more complicated. For if the top of the platform should be displaced towards the moored ship, the restoring force will simply be the buoyancy, but if the displacement is in the other direction, the force constant will be increased by the elasticity of the mooring lines.

The result is a bilinear oscillator — in each half of the range of some variable X (which is a function of the time t), the motion is governed by a second order differential equation of the usual form but with different parameters. The free oscillations of such a system are easily dealt with — simply write down the general

analytical solutions for greater than and less than zero and match them (by velocity) in the middle. Whatever the initial conditions, the outcome is a periodic motion whose frequency is the harmonic mean of free oscillations in the two separate regimes. Damping is a complication, as a little algebra will show, while the problem of vibrations driven, for example, by wave motion in the marine environment makes the search for explicit analytical solutions impracticable.

Thompson's computer program is an all-singing all-dancing affair. The case of a ship tethered to a jetty is dealt with simply by supposing that at each impact it bounces back with the same velocity. The mechanical equations are put into a non-dimensional form, in which case the behaviour of each bilinear system can be specified by three parameters representing damping, the ratio of the two frequencies in the two halves of the free bilinear system and the forcing frequency (again in terms of the free bilinear frequency). There is, of course, a doubly infinite range of starting conditions — position and velocity — to play with. The computer program is designed to spot periodic solutions automatically, by the condition that both the position and velocity of the object are regularly repeated. In part, the outcome is what might be expected — if the forcing frequency coincides with the bilinear frequency, resonance oscillations are excited. Not surprisingly, resonance oscillations of the unsymmetrically tethered system also appear if the forcing frequency is some integral multiple of the underlying bilinear frequency, but less conspicuously. For the tethered ship, the sixth sub-harmonic can be seen. Thompson has little to say about the resonance responses that appear on the other side of the fundamental response, at forcing frequencies that are irregularly sub-multiples of the underlying bilinear frequency.

Where does chaos enter this hitherto unsurprising tale? The first hint of something odd is that periodic solutions with different frequencies can apparently coexist at one and the same ratio between the forcing and basic bilinear frequency. Different initial conditions give different patterns of response. For the case of the tethered ship, for example, oscillations at the fundamental frequency of the free bilinear oscillation are to be found even when the predominant response of the system is that of the fourth sub-harmonic. This much can be checked

by plotting on a graph the position and velocity of the oscillating object at intervals of the fundamental period. The fundamental oscillation is represented by just one point in such a phase space, the second sub-harmonic by two and so on. Thompson shows that variation of the parameters may provoke the kind of doubling of the period of an oscillating system now widely recognized as a prelude to chaotic behaviour. And for his version of the problem of the tethered ship, Thompson finds some values of the parameters describing his system in which the motion is not stable at all, but in which it appears to hunt unsuccessfully between different sub-harmonics.

That these circumstances correspond to what is technically known as chaos follows easily enough. Thompson shows that period-doubling accumulates as his parameters are varied, eventually becoming infinite. One curious feature of the results is that within this regime, there are particular narrow ranges of the parameters in which apparently regular periodic oscillations can occur — but whether they do must depend on a particular choice of starting conditions. Another demonstration that these systems are chaotic follows from computer calculations showing that the difference between the trajectories with different initial conditions may diverge exponentially even when the initial conditions are infinitesimally close together. This is that property of chaos from which ultimately derives one of its most surprising features — the recognition that the sets of points (say the sets of values of initial conditions) leading to chaos may have fractional dimensions.

The importance of Thompson's calculations is, however, more immediate. First, it is not possible to identify all the possible motions of a mechanical system by starting with some simple periodic solution and changing it a little, perhaps by a perturbation calculation. Nor is it possible to recover all possible solutions by a judicious variation of initial conditions. The trouble, Thompson points out, is that most analyses of mechanical systems carried out with the help of computer programs are at present based on the supposition that small variations in the quantities specifying a mechanical system yield small changes in its behaviour. In at least one set of practical circumstances, Thompson shows this supposition to be incorrect.

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RNA viruses

Reverse transcription in plants?

from Harold Varmus

Two decades have now passed since Temin proposed that genetic information flows from RNA to DNA during replication of RNA tumour viruses¹; yet whenever encountered in a new context, the notion continues to generate excitement. In the past couple of years there has been evidence — some strong, some less than conclusive — for reverse transcription in the replication of the hepatitis B-type viruses (HBVs)², in the generation of certain mammalian pseudogenes and highly reiterated sequences³⁻⁶, and in the transposition of *copia* elements of *Drosophila*^{7,8}. Now it seems more than possible that such retrograde events also occur in plants, during the replication of the cauliflower mosaic virus (CaMV)^{9,10}, a DNA virus that has attracted considerable attention recently as a candidate genetic vector¹¹.

Support for the newest claim comes largely from structural studies of encapsidated CaMV DNA and of the intracellular DNA and RNA species judged to be intermediates in the replication of CaMV¹²⁻¹⁴. The arguments are best viewed in relation to the life cycles of retroviruses and HBVs (see the figure and refs 2,15).

CaMV shares with retroviruses the capacity to produce a major RNA species that contains all the viral information, with a short sequence (R) present at both ends¹⁵. Retroviruses use host tRNA, base-paired to an 18-nucleotide sequence near the 5' end of the full-length RNA, as a primer for synthesis of the first (minus) strand of viral DNA; short polypurine tracts (PPTs) mark the priming sites for second (plus) strands.

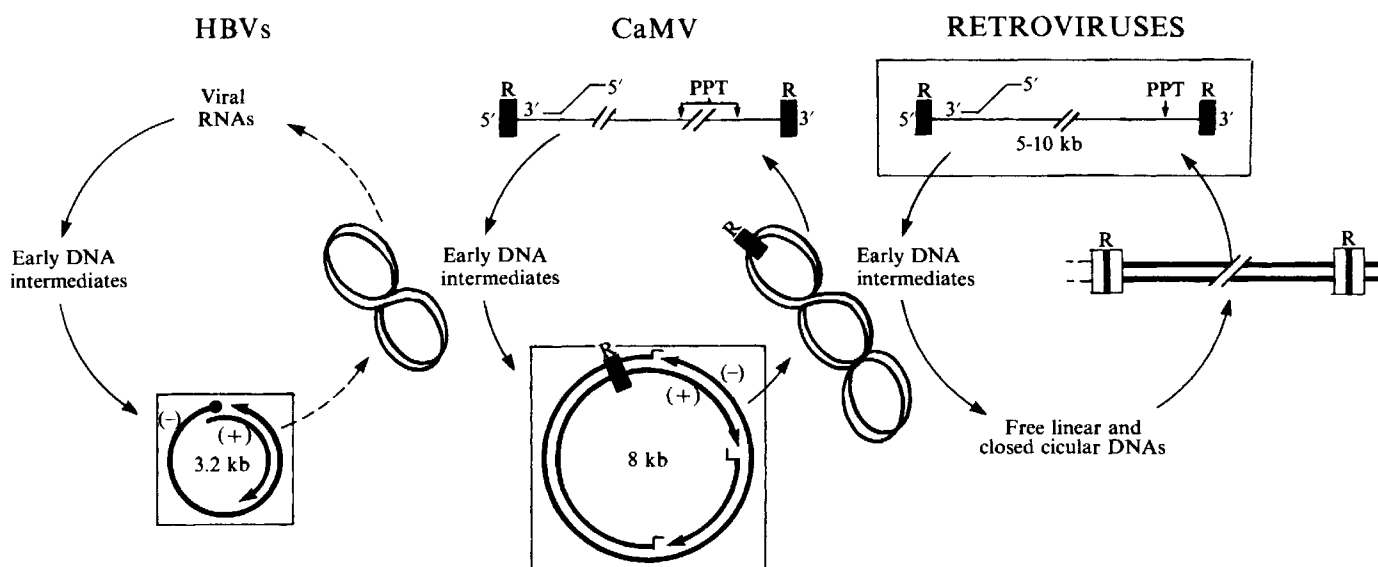
The analogous species of CaMV has now been proposed as a template for reverse transcription, in part because it contains sequences resembling retroviral priming sites: a 14-nucleotide sequence complementary to the 3' end of a plant tRNA^{Met} and short PPTs. These potential priming sites are positioned similarly to retroviral priming sites and exactly as required to produce the bizarre 8-kilobase DNA molecule found packaged in virions. This molecule, an open circle with neither strand fully circular, resembles, in turn, the genomes of HBVs, except that the HBV genomes have a large single-stranded region, because of an incomplete plus strand, whereas the CaMV genome has a few short three-stranded regions, one with a discontinuity in the minus strand and others with interruptions of the plus strand¹⁶⁻¹⁸. The third (unpaired) strand at each site appears to result from short-lived displacement synthesis, the 5' end of each displaced strand demarcating a site at which synthesis was primed. It is precisely at those sites that the sequences mimicking retroviral primers have been found.

The positions of the presumptive priming sites in relation to the ends of full-sized RNA and to each other can explain some observed DNA intermediates¹⁴ that now seem analogous to early products of retroviral reverse transcriptase¹⁵. Such intermediates appear to accumulate in both viral systems when it is necessary to transfer nascent DNA strands between templates, for example after minus-strand synthesis has reached the 5' end of the RNA

template, located a few hundred nucleotides from the tRNA-priming sites (see the figure).

Although many pieces of the puzzle are not yet in place, some important relationships are emerging from studies of these three types of virus. First and foremost, the virus particles of CaMV and HBVs contain their genes in a DNA form, whereas retroviruses carry an RNA genome. Retroviruses and CaMV seem to use RNA primers (tRNAs and polypurine oligomers), whereas at least one of the HBV strands seems likely to be primed with a protein found covalently joined to the end of the minus strand of virion DNA¹⁹⁻²¹. Retroviruses make terminally redundant RNA from an integrated DNA template that is more extensively redundant than the RNA¹⁵; CaMV seems to generate such RNA by initiating and polyadenylating transcripts at positions 180 nucleotides apart on a non-redundant closed-circular DNA template^{12,13,22,23}. In both cases, the sequence used as a polyadenylation site must first be transcribed without being polyadenylated. Though much less is known about the synthesis of HBV RNA, closed-circular DNA is found regularly in infected tissues^{24,25}, and integrated DNA suited to act as a transcriptional template is not; hence it is attractive to predict that HBV RNA, like CaMV RNA, is made from a closed-circular template.

The sharing of biochemical features runs counter to the physical appearance and modes of maturation of the three types of virus: retroviruses are large and acquire an



Proposed life cycles for hepatitis B-type viruses (HBVs), cauliflower mosaic virus (CaMV) and retroviruses as discussed in the text. R, short sequence at both ends of viral RNA; PPT, polypurine tract; ●, protein linked to 5' end of HBV minus strand; —, tRNA primers base-paired to RNA; boxed intermediates are present in mature virions; thin lines denote RNA, thick lines DNA and dashed arrows indicate uncertainty.

envelope by budding from the plasma membrane; HBVs are considerably smaller, with a glycosylated surface protein that can be independently secreted; and CaMV is a naked icosahedral nucleocapsid that accumulates in the intracellular inclusion bodies. On the other hand, the genetic organization of these viruses reinforces conviction in their similarities: in each case, all the known coding information is present in the single RNA strand thought to be used as template for DNA synthesis. Is it possible that the three classes have evolved from a common virus or cellular element, adopting very different strategies for entering and leaving host cells?

Before such elusive notions are considered, however, some straightforward enzymology is needed to place the new view of the CaMV life cycle on firmer ground. Unlike the situation with retroviruses or HBVs, DNA polymerase activity has not been described in mature CaMV particles, though there is recent rudimentary evidence for the predicted activities in extracts of infected leaves¹⁰. It is also not yet apparent whether the predicted enzyme is likely to be virus-encoded; however, most CaMV-coding domains are still unassigned¹¹. It is difficult to imagine a more inviting target for a restless enzymologist. □

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Animal behaviour

From Skinner box to the field

from John R. Krebs

TEN years ago operant psychology and behavioural ecology would have seemed most improbable bedfellows; but a recent meeting* bringing practitioners of the two disciplines together showed that after all, *afficionados* of the Skinner box and field workers peering through binoculars at foraging animals in the wild are studying essentially similar problems. Operant psychologists have concerned themselves with elucidating the behavioural rules governing the relationship between reinforcement (often food) and response while behavioural ecologists have attempted to formulate and test general rules governing choice made by foraging animals between different kinds of prey or between differing feeding sites. There is an obvious parallel between choice by a pigeon in a Skinner box with two keys, one offering a food reward every 10 pecks and the other a reward every 100 pecks, and the choice of a wild pigeon to feed in a field replete with newly sown barley instead of a nearby field with a scattering of natural grass seed. There are good grounds for expecting the rules of choice to be similar in the two contexts, and exactly how similar was one of the discussion points of the meeting.

An important difference emerged in the kinds of models of choice developed by operant psychologists and behavioural ecologists. The former are interested in describing at the behavioural level the proximate rules or immediate criteria governing choice, while the latter model choice by trying to deduce from evolutionary first principles what the criteria of choice ought to be if the individual is to survive and do well in its natural environment. The behavioural ecologist might account for the pigeon's choice of the barley field or the high reward-rate key in the Skinner box by postulating that animals are designed by natural selection to forage efficiently, since efficient foraging ultimately reflects itself in reproductive success and survival. A mathematical model along these lines might be based on the hypothesis that choice maximizes overall rate of food intake, subject to some specified constraints. The operant psychologist's approach is to investigate the nature of immediate rules guiding the pigeon's choice — for example the pigeon might be influenced by the immediate probability of reinforcement at the two sites or by the rate over a short time period. The two kinds of explanation are not, of course, mutually exclusive and one might expect the animal's proximate rule for responding to be one which ultimately produces an adaptive outcome from the evolutionary stand-point.

A response rule discussed extensively at the meeting, first proposed by R.J. Herrnstein and W. Vaughan (in *Limits to Action*; ed. Staddon, J.E.R.; Academic, New York, 1980), is melioration, choosing the alternative with the higher (or highest in multiple choice) local rate of reinforcement. Given a standard criterion for defining 'local rate of reinforcement' melioration is quite successful in describing choice for food rewards offered on different schedules in a Skinner box. From the point of view of the behavioural ecologist it is interesting to note that an animal using melioration as its choice rule often ends up maximizing overall rate of reinforcement (or coming close to this) which is equivalent to the choice criterion of many ecological models of foraging. Experiments in which melioration does not lead to overall maximizing tend to be ones in which the animal is set a highly artificial choice task (for example, pecking at key A to get rewards from site B), in which the simple rule of thumb does not do well (Mazur, J.E. *Science* **214**, 823; 1981).

The complexities of food choice in the real world were illustrated by T.C. Moermond's and J. Denslow's (University of Wisconsin) account of neotropical frugivorous birds such as the genera *Euphonia* and *Manacus*. When tested in captivity with local fruits in Costa Rica the birds show transitive preferences in pair-wise choices (A>B>C) based on sugar concentration (high > low) or size (big > small). However when a large or sugar-rich fruit is made less accessible by hanging it below a perch, the bird's preference switches: rather than hang at full stretch below the perch to eat the big one (which they are physically capable of doing) the birds take the small fruit. Moermond and Denslow suggested that the energy- or nutrient-based preference is overcome because the birds avoid putting themselves in the vulnerable upside-down position. Psychologists such as R.J. Herrnstein (Harvard University) pointed out that these results could equally well be described by saying that the birds' choices are based on melioration and hanging the fruit below the branch decreases its reinforcement value. The problem with both accounts is that they are based on *post hoc* rationalization of the data. By careful analysis of costs and benefits in the field, behavioural ecologists hope that it may be possible to come up with *a priori* accounts of the rules of choice, based on functional considerations. □

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*The sixth Harvard Symposium on Quantitative Analyses of Behaviour was held on 10–11 June 1983.

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Neurobiology

Contractile proteins in brain cells

from Richard Cumming and Robert Burgoyne

IN the search for cellular mechanisms underlying the storage of memory, the mushroom-like projections on dendrites, the dendritic spines have often attracted speculation. In the cerebral cortex 80–90 per cent of excitatory synapses are on dendritic spines and theoretical models have suggested that a modification of synaptic efficacy may result from changes in their shape or even number¹. Furthermore, alterations in spine shape have been reported following changes in environment, sensory input and long-term potentiation in the hippocampus (this last being an experimental model of long-term memory). It has recently been hypothesized that ultra-short-term memory could involve rapid 'twitching' of the dendritic spine². But have spines any molecular mechanism for changing their shape? Several recent reports suggest that they may.

The classical contractile proteins are actin and myosin, and actin has now been visualized in dendritic spines in two cases using immunocytochemistry^{3,4}, and in a third by labelling with S-1 myosin subfragment which decorates actin filaments⁵. Several interesting points arise from the immunocytochemical studies. Using an antibody against fish muscle actin, Matus' group³ reported high concentrations of actin in spines, while the main bulk of the dendrites were generally unlabelled. In the study by Cacares *et al.*⁴, two monoclonal antibodies against quail muscle actin were used, one which stained spines and dendrites in hippocampus and cortex and another which only stained dendrites; both antibodies detected brain actin by immunoblotting. These apparent discrepancies could be explained by isoforms of actin having a differential distribution in the spine and parent dendrite.

Could other cytoskeletal proteins have a role in altering the shape of dendritic spines? Microtubules may play a part in the development of the spine and its unique organelle the spine apparatus⁶, but they are only occasionally observed in adult spines though they are ubiquitous in dendrites. Although β -tubulin subunit has not been detected in spines, rabbit anti-tubulin antibodies have been found to decorate cortical spines⁷. Rather surprisingly, the microtubule-associated protein (MAP) MAP2 was widely localized in spines by Cacares *et al.*⁴. Together with an earlier report that MAPs may be present in dendrites before the appearance of tubulin or assembled microtubules during development⁸, this suggests that MAP2 may have a function unrelated to microtubule

assembly in the dendritic spine. Since MAP2 can interact with actin *in vivo* to form gels⁹, MAP2 might regulate the gel-sol transition of actin in the spine, thereby modifying spine shape. Such an interaction is likely to depend on calcium influx (as are the mechanisms generally involved in regulating cell shape) following synaptic activation. This process might well be regulated by the spine apparatus which has recently been shown to sequester calcium^{10,11}.

Many questions remain to be answered, however: what is the precise localization of these cytoskeletal proteins in the spine and is actin present in filamentous or in soluble globular form? The immunocytochemical techniques described have poor subcellular resolution, and labelling with myosin subfragment could artefactually induce polymerization of globular actin into

filaments. Perhaps clues will come from the combination of immunocytochemistry with rapid-freezing and deep-etching techniques¹².

It is clear that the dendritic spine cannot be regarded simply as an extension of its parent dendrite: however, the task of proving that cytoskeletal proteins actually have a functional role is sure to set a few spines twitching. □

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Hominid evolution

Dating Tautavel man

from A.G. Wintle

THE dating of European Middle Pleistocene hominid remains is a highly controversial affair¹. This is particularly true when physical methods are stretched to their limits, and determinations are made on materials whose properties are not yet totally understood. Two papers^{2,3} in this week's issue of *Nature* illustrate the difficulties that can be encountered.

The latest site to enter the arena is the cave of l'Arago at Tautavel in the eastern Pyrenees, France⁴. In the cave about twelve archaeological horizons have been identified in sands separated by horizontal stalagmitic floors. Systematic excavations have yielded a considerable number of bones, both animal and hominid, including a skull. The hominid bones show characteristics of *Homo erectus* but the faunal evidence is inconclusive and two different biostratigraphical chronologies have been proposed. It would seem likely that the application of one or more physically based absolute dating techniques should make it possible to fit the Arago remains into the general framework of hominid evolution in Europe.

Most of the dating at Tautavel has been carried out on stalagmitic floors since the techniques are destructive and no hominid bone fragments can be spared. Age determinations by uranium series dating have been attempted both on the basal stalagmitic floor and on travertines from the

uppermost sediments that have archaeological importance (see ref. 1 for a review). The basal stalagmite turns out to be too old to be dated by this method, which has an upper limit of 350 kyr because it depends on the build-up of ²³⁰Th towards its equilibrium value in the stalagmite. The travertines gave ages of between 100 and 300 kyr, but because they tended to be heavily contaminated considerable correction had to be made for detrital thorium.

Two other physical dating techniques that have been applied to calcite from Arago are thermoluminescence (TL) and electron spin resonance (ESR) and which are rigorously examined in this issue of *Nature*^{2,3}. Earlier results using these two methods gave ages ranging from about 200 kyr for travertines from the bottom of the upper archaeological unit (ensemble supérieur IV) to 450 kyr for the basal stalagmitic floor⁵. The TL and ESR results (using the h_3 signal) seemed to be in reasonable agreement and it was thought that any discrepancies could be accounted for by errors in the dose-rate measurements resulting from the complicated dosimetry at the site.

However more recent TL and ESR experiments have produced older ages. Yokoyama *et al.*^{6,7} have produced ESR dates of about 500 kyr for the travertine floor from ensemble supérieur IV and about 700 kyr for the basal stalagmitic floor. The main

archaeological horizons are in ensemble III beneath the travertine floor and hence an age of about 500 kyr has been proposed for the hominid remains.

Yokoyama *et al.* used a new ESR technique which involves heating the samples before measurement. The technique has been applied empirically to stalagmitic samples from Arago and another French cave site, but no comprehensive study has been made of well dated calcite. In this issue of *Nature*, Skinner³ questions the use of the h_1 signal after heat treatment in the light of her experience with a younger sample for which an age has been obtained by the uranium series technique. Instead, she proposes that the h_3 signal in unheated samples continue to be used for ESR dating analyses.

ESR and TL are two related phenomena. They both give signals which arise because of trapped electrons in the calcite lattice. Each method has its advantages and disadvantages. In TL the trapped electrons are measured by observing the light produced as the sample is heated and the temperature at which this emission occurs allows one to estimate the stability of the electrons over archaeological time. In ESR the concentration of paramagnetic electrons is measured directly by absorption of microwave energy in a strong magnetic field at ambient temperature; this measurement alone does not give any indication of the stability of the various ESR signals observed. However, the effect of heat on the ESR signal has been studied⁸.

Several attempts have further been made to correlate the h_3 ESR signal with one of the TL emission peaks (280°C) and hence to apply the results of TL stability tests to ESR data. Debenham² shows that this correlation does not hold for the samples from Arago. He further shows that the results obtained for both peaks (280°C and 350°C) are in agreement with each other and indicate that for the basal stalagmitic floor the 280°C TL signal cannot have lost more than 15 per cent of its trapped electron population. This refutes the claim of Yokoyama *et al.* that the TL dates obtained using the 280°C peak are too young because of thermal annealing of the trapped electrons at ambient temperature.

The dating of hominid remains is an emotive issue, so I doubt that this is the last that will be heard about the dating of l'Homme de Tautavel. □

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Astronomy

How to say Halley

from David W. Hughes

HALLEY'S comet has already been recovered and will return to the vicinity of the Sun at the beginning of 1986, which will obviously arouse considerable interest. But how should we pronounce the name? There seem to be three possibilities: Ha'li which rhymes with 'valley'; Hö'li which rhymes with 'poorly'; and Hā'li which rhymes with 'bailey'. The first is the common pronunciation, used by over 95 per cent of people today¹. The last is probably wrong and is a relic of the 1950s when Bill Haley dominated the pop music charts with his rock-and-roll group known as 'The Comets'. The second one was favoured by Eugene F. MacPike and more recently by Colin Ronan. They contend that it is how Edmond Halley would have pronounced his own name, living as he did between 1656 and 1742.

The main evidence comes from the way in which the name was spelt by the people who knew Halley and wrote to him. Remember that, in those days, before dictionaries, there was no such thing as the 'correct' way of spelling a word. In fact spelling was so haphazard that people would spell their names as the fancy took them at the moment. Unfortunately the old chroniclers spelt the name in many ways² — as 'Hailey', 'Haley', 'Halley', 'Hally',

'Haly', 'Hawley', 'Hawly' and 'Hayley'. So how can we deduce from the spelling which pronunciation is correct or even most probable?

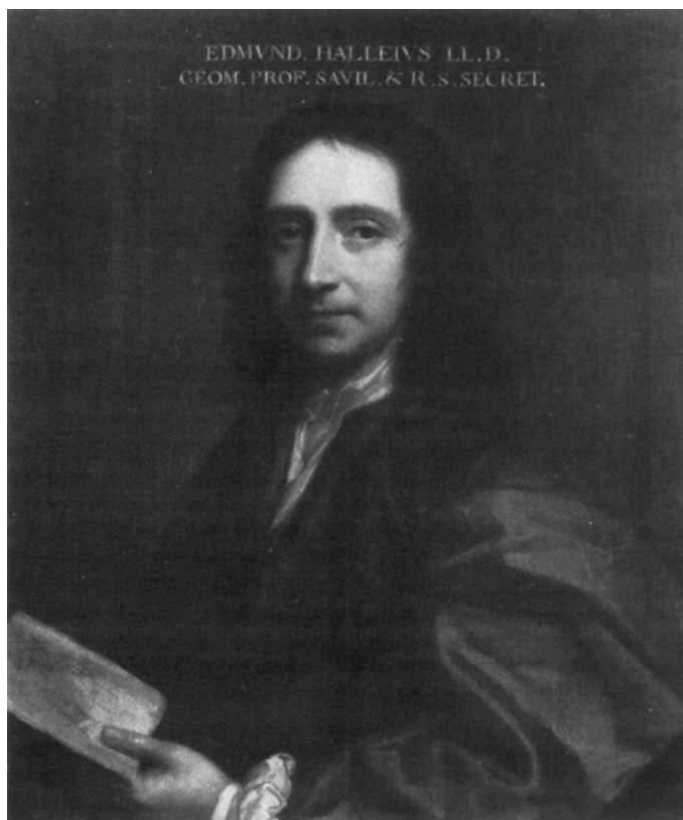
The antiquary John Aubrey (1626–97) claimed that Edmond was related to "the Halleys of Derbyshire, a good family", and this was also the opinion of John Falmstead (1646–1719), the first Astronomer Royal³. MacPike⁴ quotes one of his correspondents as writing

"I think that in the seventeenth century in Derbyshire, 'Halley' was pronounced 'Hawley' . . . There were Walleys or Whalleys in Derbyshire but I am not confident that the two families are connected."

(Wh and h were sometimes equivalent, as in who and whole, and in those days one sometimes found whot for 'hot' and whome for 'home'). Another correspondent⁴ thought that Halley, Whalley and Hawley were all variants of a specific place name. Beavor⁵ also wrote

"I think that the astronomer's name was pronounced Hawley, and we have seen that at Youlgreave the forms Halley and Hawley were used almost indifferently. But Humphrey Halley, vintner [the astronomer's grandfather], and his descendants appear to have uniformly spelled their name Halley"

Returning to Halley's contemporaries



Portrait of Halley (1656–1742) by Thomas Murray. The picture was provided by the Royal Society, of which Halley was Clerk (1696–98), Secretary (1713–21 and Vice-President (1729–? and 1741–2).

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8. Hennig, G.J. & Grün, R. *Quat. Sci. Rev.* (in preparation).

one can read⁶ "Mr. Hawly to be pd 100ⁱ to buy instruments . . .", whereas about twenty years earlier the name was spelt 'Hailey' in the register entry for his marriage at St James's, Duke's Place, without Aldgate on 20 April 1682. Halley, Haley

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6. *Calendar of Treasury Papers, 1697-1701/2* Vol. 56, no. 31, 210 (Redington, London, 1871).
7. Aubrey, J. *Brief Lives*, i.282, 283 (Clark, Oxford, 1898).
8. Quoted by Willey Ley in *Watchers of the Sky* (Sidgwick & Jackson, London, 1963).

and Haly all occurred in Aubrey's *Brief Lives*⁷.

So where does this leave us today? The name will soon be on everyone's lips and advice is needed. Maybe it is best to concur with the modern bearers of the name and follow Sir Harold Spencer Jones⁸ who regaled us with the doggerel

"Of all the comets in the sky,
There's none like Comet Halley.
We see it with the naked eye,
And periodically." □

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Neurobiology

Peptides and the microregulation of blood flow in the brain

from James McCulloch

THE survival of the central nervous system is dependent on the continuous delivery of substrates by the blood. If the supply of blood to the brain is discontinued for more than a few minutes, cerebral tissue is irreversibly damaged. Local cerebral blood flow is normally adjusted to meet the local levels of energy generation (that is, oxidative catabolism of glucose). This local coupling between blood flow and energy metabolism has been generally considered to be maintained by the local release of products of cerebral tissue metabolism — hydrogen and potassium ions and adenosine — which have been assumed to induce cerebrovascular vasodilatation. The recent demonstration by Hendry and co-workers¹ that a single cholecystikinin-immunoreactive (CCK) neurone in the cerebral cortex makes close contact both with cortical blood vessels and with other cortical neurones, provides morphological evidence of a new mechanism by which neuronal excitability, and thus energy generation, may be linked to cerebral blood flow.

This finding provides the first compelling morphological support for the hypothesis, developed over the past decade, that neurotransmitters in the central nervous system may be released from a single neurone in proximity to cerebral blood vessels, as well as at synapses with other neuronal systems. The observations that originally gave rise to the idea were that noradrenergic fibres originating in the locus coeruleus and serotonin-containing neurones arising from the raphe complex are associated with intraparenchymal arterioles and capillaries²⁻⁴. However, only for capillaries (and not for arterioles) in highly functionally specialized regions, such as the paraventricular nucleus of the hypothalamus, is there compelling ultrastructural evidence of direct contact with monoaminergic nerve fibres⁵. This

makes the photomicrographs and electron micrographs of Hendry *et al.*¹ particularly exciting, as they demonstrate that a single CCK perikaryon in the neocortex can give rise to processes, some of which appear to form synaptic contacts with other cortical neurones, and others which have truly intimate contacts with intracerebral both capillaries and arterioles. Moreover, this form of peptidergic innervation of cerebral arterioles may not be peculiar to the octapeptide CCK: there have been tentative suggestions that at least a portion of the vasoactive intestinal polypeptide-immunoreactive (VIP) fibres which innervate pial arterioles on the cortical surface arise from cortical VIP neurones⁶.

The CCK processes in contact with intracortical blood vessels are quite distinct from the classically described nerve fibres containing noradrenaline and acetylcholine, which innervate predominantly the major cerebral arteries and pial arterioles on the cortical surface⁷. These perivascular noradrenergic and cholinergic fibres, like the recently discovered fibres containing neuropeptide Y and substance P, are derived from sympathetic ganglia or branches of the cranial nerves⁸⁻¹⁰. Furthermore, there is little reliable evidence that these extracerebral peptidergic and aminergic fibres are responsible for coupling local blood supply to local metabolic demand in cerebral tissue.

Other than the immunocytochemical data^{1,6}, there is only the most circumstantial evidence implicating polypeptides such as CCK and VIP in the maintenance of the inter-relation between neuronal activity, energy generation and local blood flow. CCK and VIP certainly increase the firing rate of cortical neurones¹¹, and VIP stimulates the breakdown of glycogen *in vitro*¹² and the local phosphorylation of glucose *in vivo*¹³. VIP can elicit the dilatation of cerebral arteries and arterioles^{14,15}.

It will be of considerable interest to see whether CCK displays similar actions in these test conditions.

Caution will however have to be exercised in the interpretation of such data. VIP administration increases the overall electrical activity of the brain, with parallel increases in global cerebral blood flow and oxygen consumption¹⁵, but it is unknown whether each of these changes is initiated by VIP itself, or whether more classical coupling factors such as hydrogen and potassium ions are responsible. The availability of reliable techniques for analysing cerebrovascular reactivity *in vitro* and *in situ*, and of autoradiographically based techniques for examining, in highly discrete central nervous system regions, the relationship between glucose use and blood flow should make it possible soon to resolve these questions.

The involvement of cortical CCK neurones in the local coupling of neuronal activity to blood flow is only one of a number of possibilities raised by the work of Hendry *et al.*¹. The proximity of CCK-containing cell bodies to cerebral blood vessels and the direct contact of some CCK processes with the basal lamina of the capillaries may reflect a role for these neurones in, for example, monitoring the chemical composition of the extracellular environment or of the blood. Alternatively, CCK may modulate the permeability of the blood-brain barrier. The possibilities of co-transmission (CCK co-exists with dopamine in neurones in the ventral mesencephalon)¹⁶, and of similar anatomical arrangements in peptidergic systems other than those involving CCK and VIP, widens the range of possibilities even further. If the observations on CCK are upheld, they may have important practical implications: it is the inability of normal homeostatic mechanisms to maintain the relationship between cerebral function, metabolism and blood flow that is a crucial element in all cerebrovascular disease. □

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Reproductive immunology

Early pregnancy factor

from A. Whyte and R.B. Heap

THE way in which the maternal immune system is regulated during pregnancy to allow the survival of the fetal allograft remains unresolved. Various compounds whose concentrations in the maternal circulation are increased during pregnancy have been implicated. The hormones of pregnancy have long been suspected to have a role since they are produced in substantial amounts by the placenta of certain species resulting in high local concentrations¹, and because they can inhibit lymphocyte transformation *in vitro*^{2,3} and reduce the granulomatous reaction to a foreign body *in vivo*^{2,4}. Another candidate is early pregnancy factor (EPF), which was first described by Dr Halle Morton and her collaborators. Claims that EPF can be detected very early in pregnancy, that abortion coincides with loss of activity, and the ability of EPF to inhibit certain immune reactions all support an immunoregulatory role for this factor during pregnancy. Confirmation of the claims has in some cases proved difficult however, and nearly ten years after its discovery the physiological role of EPF is still far from clear.

EPF was first described by Morton and her colleagues in *Nature* in 1974. Their experiments were based on the rosette-inhibition test which was originally described by Bach and Antione⁵ as a means of assessing the activity of anti-lymphocyte serum (ALS). The test depends on the ability of sheep red blood cells (SRBCs) spontaneously to form clusters (rosettes) around human T lymphocytes. Addition of ALS 'sensitizes' the lymphocytes and, in the presence of complement, inhibits rosette formation. Morton *et al.* observed that the dilution of ALS able to reduce rosette formation between mouse spleen cells and human red blood cells to 75 per cent or less of the control — the rosette inhibition titre (RIT) — was much higher when spleen cells were taken from pregnant female mice compared with non-pregnant mice⁶. Furthermore, the incubation of lymphocytes from non-pregnant females in serum from pregnant (but not non-pregnant) females also reduced the amount of ALS that was required to inhibit rosette formation, indicating that the active compound, EPF, was present in serum of pregnant females⁷.

It is claimed that EPF can be detected very early in pregnancy. Using the rosette-inhibition test, the serum factor has been detected in mice from 6 h after mating⁷, and in women soon after fertilization with activity being lost after surgical abortion, or declining before spontaneous abortion^{8,9}. EPF has also been reported to be present in the sera of some women using intra-uterine contraceptive devices (IUDs) 6–8 days after a presumed ovulation, but

not in women using other forms of contraception¹⁰. These results were taken to confirm that although fertilization may occur in women using IUDs, implantation and subsequent embryo development is prevented. But in none of these studies was the presence of a conceptus confirmed by independent tests.

In sheep and pigs the RIT has been used to detect fertilization, to monitor the course of early pregnancy and to study the onset of early embryonic death^{11–16}. However, in sheep the lack of control data from pregnant animals leaves unanswered the question of whether a decline in the value of the RIT is related solely to embryo loss or also to the stage of gestation^{11,16}.

Some attempts have been made to study the characteristics and origin of EPF. Mouse EPF exists in serum in two forms, one of high molecular weight (~180,000) being replaced as gestation proceeds by a lower-molecular-weight form (~40,000)¹⁷. Three forms of sheep EPF have been described (250,000, 50,000 and 20,000 molecular weight) and part of the variation has been attributed to an association of EPF with carrier proteins¹⁸.

The different forms of EPF may arise by various combinations of two components, A and B, which are separable by ammonium sulphate precipitation. In the mouse, component A is formed in the oviduct during oestrus and pregnancy; whereas component B is apparently formed in the ovary following mating. The production of component B can be initiated in the ovaries of non-pregnant mice by fertilized ova when incubated in the presence of pituitary extracts of oestrous, dioestrous or pregnant mice¹⁹. Another factor, ovum factor (OF)²⁰, which is produced by the fertilized ovum after penetration by the spermatozoon, also seems to be involved as it has been shown to stimulate the production of component B in the ovary, probably after interacting with prolactin.

Attempts by independent groups to confirm the early production of EPF have proved largely unsuccessful^{21,22}. This lack of confirmation is disturbing, and casts some doubt on the validity of the rosette-inhibition assay for EPF, on which all the early claims were based.

These doubts have arisen for three main reasons. First, an adequate explanation for the mechanism by which EPF affects the RIT is lacking. The total rosetting T-cell population requires overnight incubation and at least two sub-populations are involved, one of which forms 'early' rosettes within 5 min of exposure to SRBCs. It seems that EPF potentiates the inhibitory action of ALS only on the early-rosetting

T-cells whose immunological function is unknown. When rosette formation is enhanced, as with bovine fetal serum²¹, the response to EPF is diminished or lost²³.

Second, the test is a multifactorial assay subject to many technical pitfalls, including differing activities of various batches of ALS and guinea-pig complement; the remarkably low titre of ALS at which the test is performed (probably less than 10 molecules of immunoglobulin for each lymphocyte); and problems derived from differences in the source, age and treatment of SRBCs²⁴. Thus failure to confirm the results of Morton and her co-workers may result from differences in technique, rather than disprove the existence of EPF. For example, Thomson *et al.* used anti-lymphocyte globulin (ALG) raised against peripheral lymphocytes²¹, whereas Morton *et al.* originally used ALS raised against neonatal thymocytes⁸ which may contain antibodies not present in ALG. The great variations that have been found between batches of ALS in the detection of EPF suggest that specificity is extremely important probably because there is an antibody in ALS which is specific for a unique cell-surface antigenic determinant. This hypothesis is supported by the fact that mouse monoclonal anti-Ly₁, but not anti-Ly₂, can be substituted for ALS in the rosette-inhibition test for EPF. Monoclonal anti-human Lyt3 has a similar effect²⁵.

A further complication with the assay is the possibility that the activity measured is not a pregnancy factor but rather auto-rosette inhibition factor^{25,26} which is present in normal serum, increases in fetal

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serum during development and, like EPF, is not species-specific. Clearly, a specific immunoassay for EPF is a high priority.

An important question is whether EPF regulates immune function. Adoptive transfer of contact hypersensitivity to trinitrochlorobenzene (TNCB) by lymphoid cells from sensitized mice was suppressed by serum fractions (from pregnant sheep) containing EPF activity. Delayed cutaneous hypersensitivity (DCH) was much reduced if TNCB-sensitized lymph node cells were incubated in these fractions before transfer into naïve syngeneic mice²⁷. Adoptive transfer of contact hypersensitivity is thought to be T-cell dependent and it may be that the active-rosetting T-cell population is similar to the T cells involved in DCH reactions²⁸. Thus the *in vitro* assay for EPF could be detecting a sub-population of T cells which does appear to have an immunological role *in vivo*. EPF may also be one of the contaminants responsible for the inhibition of lymphocyte transformation *in vitro*²⁹ claimed for certain

crude preparations of human chorionic gonadotropin.

Clearly, a healthy scepticism about the function (and nature) of EPF will persist until both a reference compound (let alone a purified compound) and a reliable *in vitro* assay are available. The physiological requirement for EPF is debatable since mouse eggs fertilized and cultured to the blastocyst stage entirely *in vitro* give rise to normal pregnancies after transfer to non-pregnant females not previously exposed to EPF³⁰. Moreover, a recent study implies that the occurrence of EPF (or a related compound) may not be confined to females since activity was detected in patients with germ-cell tumours of the testis, but not in those with non-germ-cell tumours or benign testicular disease³¹. Is EPF yet another member of the growing number of onco-developmental proteins? □

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Soil analysis

Mapping human activity in soil

from Peter D. Moore

THE abundance of phosphorus in soils is often very variable even over relatively small areas, and this is particularly noticeable where there is a local concentration of human activity. One of the most lucid demonstrations of this fact is the survey performed by Streeter¹ on Box Hill in Surrey, England. The site is part of the chalk escarpment of the North Downs, within easy travelling distance of London and hence much frequented by urban dwellers seeking rural landscapes. The chalk soils of the area are poor in phosphorus, having levels often less than 0.001 per cent P₂O₅ using citric acid extract, but Streeter found high spatial variability in one area which was intensively used for recreation.

A stone-built viewing platform forms the focal point of the survey area and phosphorus levels around the base of the platform were as high as 0.05 per cent. In general the levels diminished with distance from the platform, but erratic peaks of up to 0.015 per cent occurred, probably because of very local soil eutrophication as a consequence of the deposition of cigarette ends, litter or animal excreta. This in turn led to modification and depauperation of the normally rich chalk flora.

The fact that human activity can produce such diverse mosaics of soil phosphorus has provided archaeologists with a technique for the detailed reconstruction of former human occupation sites. The application of soil phosphorus analysis to archaeology has been reviewed by Eidt², who claims that when phosphorus is added to a soil it is not easily removed, but accumulates and is retained *in situ* and hence

reflects the pattern of past land use. Since phosphorus can be held with varying degrees of tenacity in a soil, however, he recommends a three-stage extraction process which removes (1) loosely bound phosphorus; (2) occluded phosphorus, for example that incorporated with iron and aluminium oxides; and (3) tightly bound phosphorus as apatite or calcium phosphate. The value of this kind of fractionation is that it permits the analyst to distinguish between soils which are naturally rich in phosphorus and those which have become enriched as a result of human activity. The former will contain most of its phosphorus in the tightly bound fraction, whereas soil contaminated by man will be rich in all three fractions.

It has been possible to use soil phosphorus levels to detect micro-scale patterns of building and land use in archaeological sites. For example, Conway³ has recently used soil phosphorus analysis in the excavation and investigation of a Roman-British group of huts in North Wales and has analysed the data using trend surface analysis to clarify focal points of phosphorus accumulation and overall abundance. Conway found that domestic buildings had higher levels of phosphorus than non-domestic ones (for example, barns) and displayed greater variability of pattern within the building. Often the levels of phosphorus were higher around the walls, perhaps because of the presence of dung in the daub used for their construction. The variability was most apparent in the extractable (loosely bound) fraction rather than total phosphorus, again confir-

ming that the phosphorus had its origin in external additions to the soil rather than from natural weathering.

As well as being applicable to the detection of small-scale variations within a small (in the above example, ~30 m × 30 m) plot where the general pattern of buildings has been well documented by excavation, soil phosphorus analysis can be applied to cases where the precise settlement pattern is unknown. For example, Konrad, Bonnichsen and Clay⁴ have performed soil analyses for four sites in Maine, North America, each of about 4,000 m². Samples were taken from points of intersection on a 4-m grid over the study areas, with intermediate samples collected in sites of high phosphorus concentration. A pattern emerged in which soil phosphorus was focused in certain small areas, which were interpreted as the sites of prehistoric domestic dwellings. Further, magnesium was found to be concentrated in certain locations which probably represented the sites of former hearths. Thus a grid of soil analyses can provide a guide to the most appropriate locations for detailed excavation.

Enthusiasm for this technique must be tempered with caution, however, since there are many possible complications. As Streeter has shown in contemporary studies, man does not need to construct a dwelling in order to enhance soil phosphorus. Regularly used prehistoric tracks and trampled areas may well be expected to have raised levels of the element, as might middens and latrines. Natural patterns of soil phosphorus variation could largely be eliminated if the analysis recommendation of Eidt were adhered to, but often the large number of samples involved has led to the adoption of simplified techniques, or the use of total soil phosphorus. Topographical and hydrological factors, such as leaching from ridges and fluting in valley bottoms, may be expected to lead to variations in phosphorus movement and concentration in soils, and thus could complicate the interpretation of data. Clearly the certain identification of an 'anthrosol' is not an easy matter, but it would be greatly assisted by further surveys of small-scale spatial variations in contemporary soil chemistry such as that conducted by Streeter. This will be a particularly valuable aspect of the current developments in experimental archaeology in which past techniques of land use and dwelling construction are being employed⁵. The effect of such experiments on soil conditions needs to be carefully monitored. □

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Vision

New depths in stereopsis

from Graeme Mitchison

BY combining the images of the two retinæ, we are able to construct a three-dimensional map of the visual world. This process, called stereopsis, depends upon simple geometry: the angle between the lines of sight from the two retinæ to a point varies with the distance of that point. Thus, given the directions of gaze of the eyes, the distance of a point can be inferred from the difference between the positions of its image on the two retinæ — the horizontal disparity. One might then suppose that a depth map could be constructed by identifying an object in each eye's view and calculating horizontal disparities. A striking challenge to this naive notion came from Julesz's¹ invention, two decades ago, of the random-dot stereogram (RDS). Here, a set of dots, randomly distributed on a two-dimensional sheet, is presented to one eye, while the other eye is shown the same pattern of dots, but with displacements of individual dots to give suitable horizontal disparities. In certain conditions, a three-dimensional image is perceived, and we may say that the stereogram has been 'resolved'. As the image for each eye is in itself meaningless, disparities must be inferred from the matching of more primitive features than identified objects. The nature of these stereoscopic primitives and how they are handled is still a matter for debate. Recent evidence from psychophysics and neurophysiology has cast some light on the subject.

Julesz originally argued that the primitives matched in a RDS must be points; that is, the visual system must somehow find which pair of points in the two images corresponds to a point in the three-dimensional realization of the RDS. He suggested that this matching could be achieved by a cooperative process which searches for the pairing which creates fewest abrupt depth changes. An algorithm based on this principle has been shown by Marr and Poggio² to be capable of resolving RDSs with reasonable success. However, Marr and Poggio later pointed out that RDSs are not without features, and they developed an algorithm³ in which characteristic clusters of dots — essentially edges of various orientations and degrees of blur — were matched in the two images. Because such clusters are sparser than the individual dots, there are fewer possible matches to be considered, and the algorithm is considerably more efficient.

It is still not clear which of these algorithms is more biologically reasonable. However, it has at least been possible to show that one class of primitive is probably not used in human stereopsis. The story begins with an intriguing experiment by Julesz and Spivack⁴. They designed a

stereogram whose components were pairs of vertical lines, one above the other, but with a tiny horizontal offset, which might be as little as 16 seconds of arc, between them. Now it is known that human subjects can identify the direction of displacement of such tiny offsets between pairs of lines, or 'verniers'. But because the separation can be far smaller than the spacing of cones in the retina, it has been suggested that some kind of special processing is needed for this task^{5,6}. Julesz and Spivack took a regular array of verniers with randomly assigned directions of offset, and introduced horizontal disparities by shifting individual verniers by a multiple of the spacing of the array. Thus the images for both eyes consisted of roughly equally spaced arrays of verniers, but only one pattern of matching would pair each vernier with another having the same direction of offset. They found that subjects could resolve these stereograms, and interpreted this as showing that the vernier breaks could be matched (with others of the same direction of offset) in parallel over the field.

However, a different interpretation has recently been given by Nishihara and Poggio⁷. To make the verniers in the stereograms, the vertical lines must be given small lateral displacements of random sign, so two verticals which lie next to one another in the array may have a slightly larger or smaller horizontal separation than the average. Nishihara and Poggio have shown that these shifts would cause appreciable changes in the responses of nerve cells of a centre-surround type (that is, cells which are excited by spots of light within a certain roughly circular region, and inhibited by spots of light in a surrounding annulus). They argue that it is not the vernier breaks that are matched, but the patterns of varying activity in such cells due to the shift of the verticals above and below each break.

If this is correct, then increasing the spacing between the verniers in the stereogram, while keeping the offset of the same size, should produce a smaller proportional change in the response from their

centre-surround fields, and should impair resolution of the stereograms. But if it is the breaks which are being matched, as Julesz and Spivack claimed, then separating the verniers should have little effect. In fact, the more widely spaced stereograms which Nishihara and Poggio constructed are far more difficult to resolve.

Of course, it should eventually be possible to understand stereopsis by examining the underlying neuronal machinery. Recently, G.F. Poggio⁸ has discovered cells in areas 17 and 18 (the first two cortical visual areas) of rhesus monkeys which respond in a disparity-selective manner to RDSs.

The monkeys were first shown (monocularly visible) bars of light against a textured background, and a cell was found which responded preferentially when this bar had a particular disparity. Next, the animals were shown RDSs (with no monocular cues), which combined to yield a three-dimensional view of a bar against a background. About one-fifth of the previously characterized cells also responded to this stimulus in a disparity-tuned manner. These cells were mostly of the complex type, as classically defined by Hubel and Wiesel⁹. Such cells respond only to an appropriately oriented edge of light, and are characterized by the fact that, to be an effective stimulus, this edge does not have to be at a particular location in visual space, but can lie anywhere within their receptive field. Poggio's cells responded best when the solid light bar stimulus had a particular orientation. Interestingly, however, a cell would generally show less orientation specificity for RDSs.

To account for the properties of these cells, Poggio⁷ has suggested that they might have numerous receptive sites, or subfields, in the receptive field for one eye 'gated' with a similar set in the other eye, with an appropriate disparity. This could be regarded as a kind of correlation-like operation on the inputs of the two eyes, with a cell responding whenever a large enough proportion of features in the receptive field for one eye was matched by features (with an appropriate disparity difference) in the field for the other eye.

What are the features which are matched? Although Poggio does not commit himself on this point, one natural possibility is that they are local edges, or small clusters of points, with a particular orientation. This would be compatible with the orientation selectivity shown for solid light bar stimuli, and also with the fact that the cells are less highly orientation-tuned to RDSs, given that such patterns will generally contain clusters of points of all orientations.

Now correlation is different in principle from Julesz's type of cooperative matching algorithm. Correlation by itself cannot produce an appropriate matching in all conditions. For example, if a RDS represents a plane at a given disparity together with a single point at a different

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disparity, then that point will often be immediately detected by a human subject. A correlation mechanism will find the plane, but not the odd point. It is therefore likely that, if these cells do carry out a correlation-like operation, it only represents one stage or aspect of the stereoscopic machinery. It might be useful for finding a crude decomposition into depth planes, after which departures from these planes could be specifically searched for, perhaps by cells with receptive fields organized to detect disparity discontinuities.

It is clear that neurophysiology, psychophysics and computational theories provide a powerful combination of techniques

for understanding stereopsis. G.F. Poggio's recent work is especially encouraging, and his conclusions about the kind of processing carried out in early visual areas are highly intriguing. One inference, as I have argued, is that one should not necessarily expect to find a single mechanism that solves the whole problem of stereopsis. Vision may be no less multifarious and accidental than other aspects of biology. □

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Cultural evolution

Anthropology and cultural transmission

from L. Cavalli-Sforza, M. Feldman, S. Dornbusch and K.-H. Chen

MARKS *et al.* have recently criticized in *Nature*¹ a paper² in which we summarized a theoretical treatment of cultural transmission and evolution (given in detail elsewhere³), giving simple examples of quantitative analysis of person-to-person transmission, to illustrate empirically a few mechanisms of cultural transmission and how they can be quantitatively analysed. The authors of the critical article¹ object to our use of the term 'culture', arguing that culture is a property only of societies, not of individuals. They consider trivial the fact that communication takes place from person to person. They are opposed to the use of quantitative or analytical techniques, and in general to models and metaphors.

There is no room here to discuss the methodological and general aspects of our different approaches, but we can list briefly a few major misreadings by our critics. According to Marks *et al.*, we have "capriciously defined" culture by not confining ourselves to the narrower definition "used by professional anthropologists". Actually, we explicitly (not capriciously) used the relevant definition in the *Third International Webster Dictionary*. In spite of another statement by these authors, we have not investigated religious "values", but simply religious behaviours and states. Further, they cite Menozzi *et al.*⁴ who interpreted a component of the geographical patterns of genes in Europe as a residual of the diffusion of farmers from the nuclear area of origin in the Middle East, as if it

were an example of cultural diffusion (at least we interpret in this way their "metaphorical" statement on mothers tricking their daughters into agriculture). In fact, we found the data supported another type of diffusion^{5,6} which was called 'demic' — that is, a spread of farmers to be contrasted with 'cultural' diffusion (the spread of farming). They maintain that in our monograph³ we did not use archaeological or ethnographic data. Their claim is false; see pages 42–43, 54–55, 67, 143–148, 167–171, 209–212, 255–266, 307–314, 319–320 and 325–332 of ref. 3. Their major error concerns what we call the transmission ratio. This is the ratio of the

number of transmitters to that of transmittes in specific transmission mechanisms, which may vary from a number much greater than one in the case of social pressure, to a number close to one in parent-child and other types of transmission, and to a very small fraction in the teacher-to-student situation, or situations of social leadership, or communication by media, and which may have different evolutionary consequences^{2,3}.

To our critics the last of these mechanisms, in which one person may determine the behaviour of a great number of others, suggests only the idea that "a bottom-heavy demographic pyramid leads to rapid cultural change". But a main reason why an excess of young individuals (if this is what they mean) may be accompanied by rapid cultural change is that when there are important influences from outside cultures producing rapid changes in the balance of birth and death rates, there are likely to be many concomitant cultural changes. These and other comments, for example "if culture is transmitted and possessed by individuals it is an analogue of the genotype" (why not the phenotype?), can only be labelled as misunderstandings. Our critics seem to have missed entirely the fact that each cultural trait that lends itself to dissection may have its own pattern of variation and transmission. By refusing to look at the trees they miss both the trees and the forest. □

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Jon Marks and Edward Staski reply:—

IN regard to the reply by Cavalli-Sforza *et al.*, is it not somewhat naïve of professional scientists to rely upon dictionary definitions of formal concepts? If we, for instance, were to rely upon *Webster's New 7th Collegiate Dictionary's* definition of 'species' as 'a class of individuals having common attributes and designated by a common name', it is unlikely that we would receive any credibility within the community of biologists. Nor would we deserve any.

It appears, however, that to Cavalli-Sforza *et al.* such naivete is acceptable, so long as unambiguous models are constructed that are capable of generating testable expectations. Expectations, however, are only useful when they mesh within a unified conceptual scheme. To discuss a topic of research — cultural evolution — that has for several decades been a focus within anthropology's conceptual scheme, one would conceivably wish to utilize the theory, methods and data in the abundant literature of anthropology.

Indeed, we reaffirm our statement that "neither in their paper nor in their longer monograph do the authors produce any

relevant archaeological or ethnographic data". If Sir James George Frazer were still considered the standard for such data, our statement would be false. Yet in the last century anthropology has exhibited progress sufficient to discredit the idea that behavioural monads may be wrenched from cultural context and still be ethnologically valuable.

We still fail to see how any evolutionary analogy which fails to distinguish between properties of the phenotype, genotype and gene pool can possibly be considered either unambiguous or an advancement. Finally, the necessary relationship between cultural transmission and cultural evolution remains difficult to see. Certainly, the principles of biological evolution were formulated decades before the principles of biological transmission; and the major features of evolution are hardly illuminated by an understanding of meiosis. □

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Age of the Plio-Pleistocene boundary in the Vrica section, southern Italy

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New palaeomagnetic data from the proposed Plio-Pleistocene boundary stratotype section (Vrica section, southern Italy) are presented here in order to improve the correlation of these sediments to the magnetic polarity time scale. If the interpretation preferred here is correct, the boundary falls above the Olduvai normal subchron at approximately 1.6 Myr.

THE term Pliocene was first introduced by Lyell^{1,2} who subdivided it into an 'older' Pliocene and a 'newer' Pliocene, later called Pleistocene. The two were originally distinguished by the percentage of living mollusca present. Since the original definition, there has been continued argument on how to recognize the Plio-Pleistocene boundary in the rock record (see ref. 3). The 18th International Geological Congress (London, 1948) agreed to place the boundary at the "first indication of climatic deterioration in the Italian Neogene succession" as evidenced by the marine fossil record. Following a 30-yr debate during which several sections came into consideration, the Vrica section in Calabria, Italy, was proposed as the boundary stratotype section⁴. Colalongo *et al.*⁵ have proposed that the Plio-Pleistocene boundary stratotype be placed in the Vrica section in coincidence with the physical horizon immediately below the first appearance of the first 'cold guest', *Cytheropteron testudo*. This proposal was accepted by the Joint Meeting of the INQUA Subcommission 1-a (Pliocene-Pleistocene boundary) held in Moscow in August 1982 and the working group of IGCP Project No. 41 (Neogene-Quaternary boundary).

The problem of recognizing the boundary outside of the proposed boundary stratotype section remains. Correlations based on first appearance datums (FAD) and last appearance datums (LAD) of suitable microfossils are at best limited to marine sequences and require the assumption of worldwide penecontemporaneity. Attempts at radiometric dating of the 'm' ash level (see Fig. 3), closely associated with the FAD of *C. testudo*, have largely failed, although an ash layer lower in the section has been successfully dated⁶. It seems, therefore, that the magnetic stratigraphy of the proposed boundary stratotype section would do much to facilitate identification of the boundary elsewhere. We now present new paleomagnetic data for the Vrica section in an attempt to tie the proposed Plio-Pleistocene boundary stratotype to the magnetic polarity time scale.

Sampling and laboratory treatment

Samples for palaeomagnetic analysis were collected using the technique described by Johnson *et al.*⁷ for sediments too soft for a standard rock drill. Three separately oriented samples were collected from each of 84 sites. Samples were collected from 49 sites in the Vrica section as described by Selli *et al.*⁴ and from an additional 35 sites in a lower section (Stuni section, located ~1.5 km east of the Vrica section), whose top is estimated by one of us (G.P.) to be less than 50 m below the bottom

of the Vrica section, based on geometric calculations using the attitude of the beds. The palaeomagnetic samples were cut into 10-cm³ specimens and all measurements were made on the SCT cryogenic magnetometer at Lamont-Doherty Geological Observatory.

The mean intensity (total moment) of the natural remanent magnetization (NRM) of the specimens was found to be $6.51 \pm 8.2 \times 10^{-6}$ EMU, several orders of magnitude above the noise level of the instrument. The NRM directions were dominated by a component of magnetization along the present field. Stepwise thermal demagnetization was carried out on at least one specimen from sites spaced at ~25-m intervals throughout the sections for a total of 22 detailed demagnetization curves. Duplicate specimens from seven of the pilot sites were subjected to alternating field (a.f.) demagnetization. Vector diagrams of the behaviour of one such pair (VA1A and VA1B) are shown in Fig. 1a, b. The other six sites behaved in a very similar manner. The NRM of both specimens is in the present field direction (north as shown by the solid symbols and down as shown by the open symbols). Specimen VA1A, after heating to 200 °C and cooling in field free space, had a reversed magnetization. Although the subsequent decay can hardly be called linear, we believe there is a single component of magnetization in a reversed direction, but that the specimen intensity, after demagnetization, is close to the noise level of the instrument and the direction is therefore difficult to measure accurately. Specimen VA1B, the twin of VA1A, was subjected to a.f. demagnetization as shown in Fig. 1b. The specimen underwent a linear decay apparently to the origin, retaining a present field direction. After stepwise a.f. treatment to 40 mT (400 Oe), the specimen was heated to 200 °C and allowed to cool in field-free space. Although the comparative intensity is quite low, a reversed direction has been isolated. Although other specimens were subjected to a.f. treatment to 80 mT, in no case did a.f. demagnetization serve to remove the present field direction, indicating that the present field component is of higher coercivity than the primary component and that a.f. demagnetization is insufficient for successful polarity determination in these sediments.

The characteristic magnetization of the Vrica and Stuni specimens can be isolated by thermal demagnetization at temperatures ranging from 200 to 400 °C, as shown by numerous demagnetization curves. The blocking temperature of the present field component ranges from <200 °C to between 350 and 375 °C. The high coercivity and variable blocking temperature of the present field components suggest that it is carried by ultrafine grained haematite^{8,9} or maghaematite. A mixture of goethite and haematite cannot be excluded, however. The specimens themselves are not visibly red pigmented, but a yellow-

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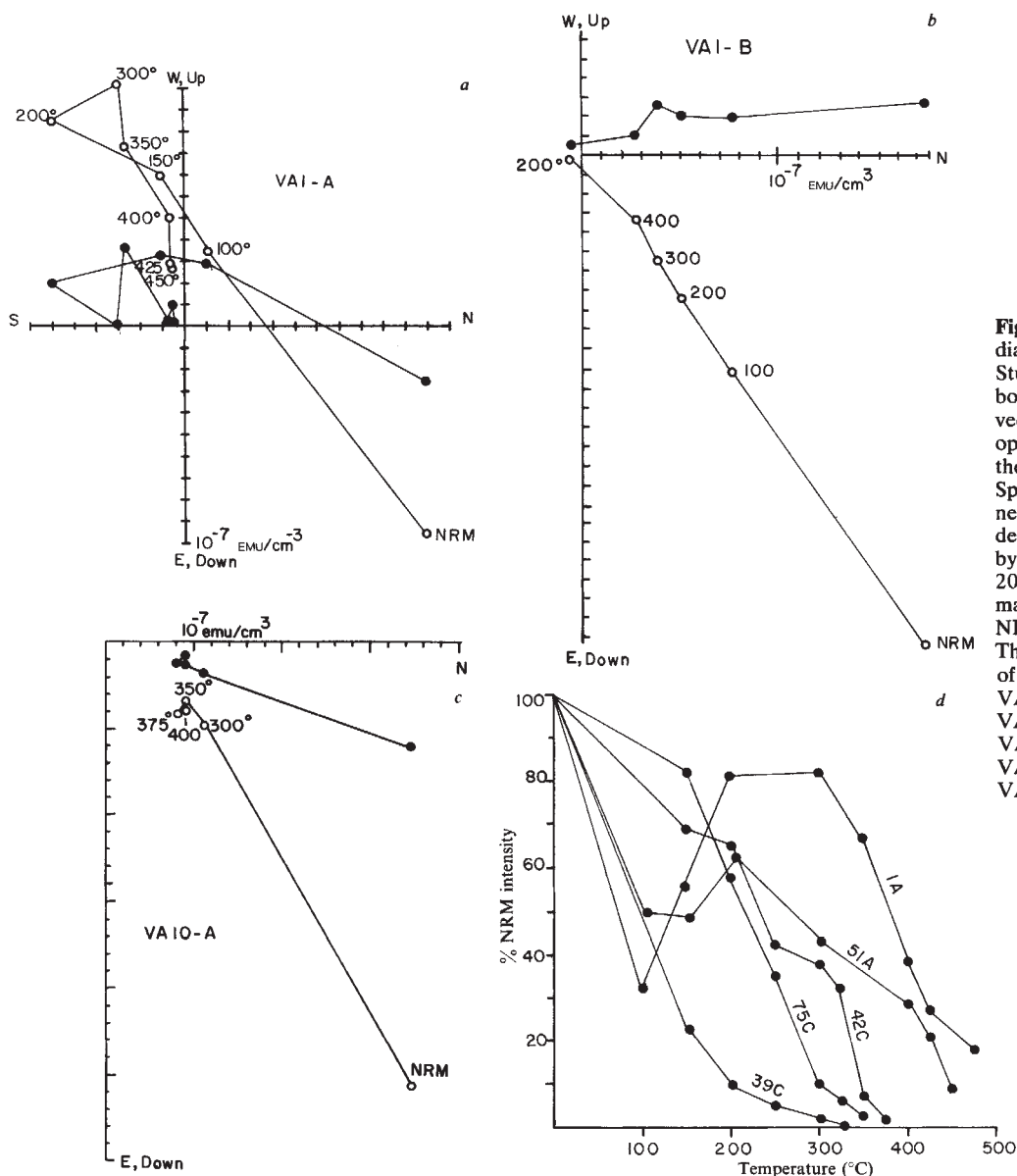


Fig. 1 Vector demagnetization diagrams of specimens from the Stuni and Vrica sections. Solid symbols are projections of the magnetic vector onto the horizontal plane, open symbols are projections onto the north-south vertical plane. *a*, Specimen VA1A, thermal demagnetization. *b*, Specimen VA1B, a.f. demagnetization to 40 mT followed by thermal demagnetization at 200 °C. *c*, Specimen VA10A, thermal demagnetization. *d*, Decay of NRM on thermal demagnetization. The NRM intensities (total moment) of the specimens are:
 VA1A, 1.13×10^{-6} EMU;
 VA39A, 5.38×10^{-6} EMU;
 VA42C, 11.82×10^{-6} EMU;
 VA51A, 2.77×10^{-6} EMU;
 VA75C, 21.02×10^{-6} EMU.

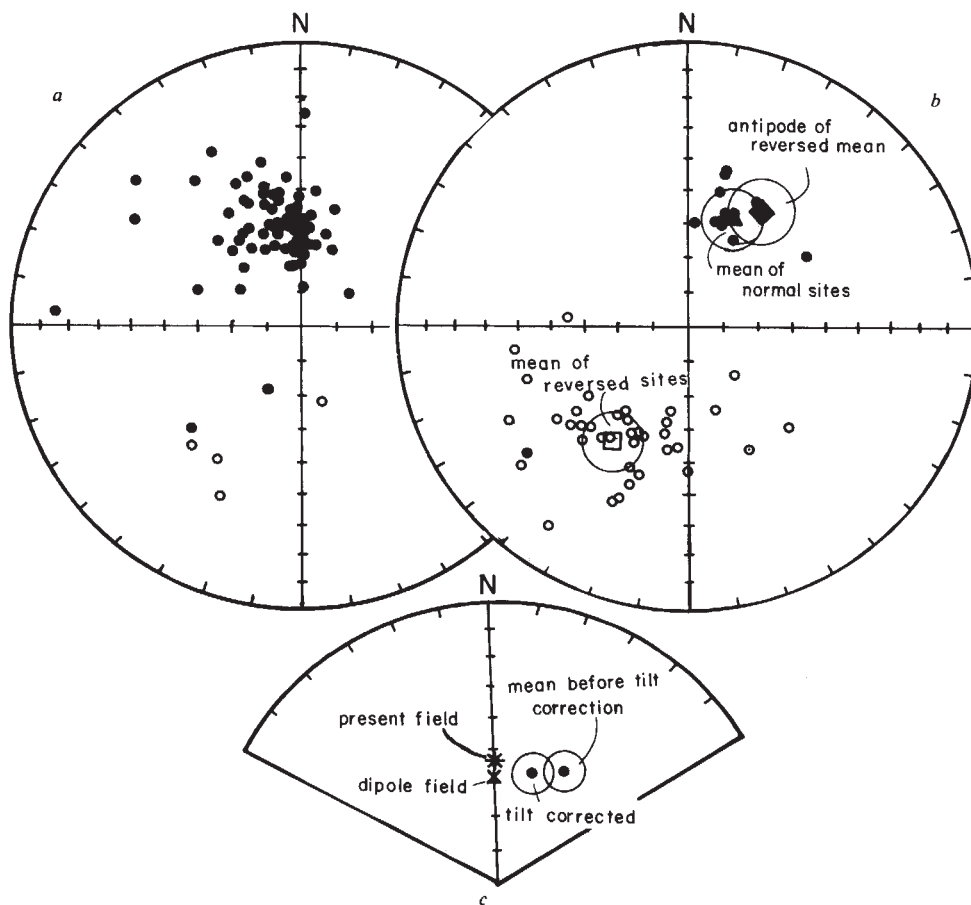
brown stain suggestive of limonite or goethite was visible in outcrop. The haematite-goethite component is clearly post-depositional in origin and the maximum blocking temperature of near 350 °C suggests that it is probably a viscous remanent magnetization (VRM) acquired in the present field direction.

The blocking temperature of the characteristic component ranges from 325 to above 500 °C (see Fig. 1*d*). This variability is believed to be caused by differing, but low, concentrations of the magnetic mineral. Consequently, measurement of the remanent intensity is limited by the sensitivity of the instrument and not by a true range of blocking temperatures. The low coercivity and maximum blocking temperature of >500 °C suggests that the characteristic component is carried by magnetite or a low titanium titanomagnetite and is therefore likely to be a primary remanence.

The combination of highly variable blocking temperatures of the present field component and the low intensity of the characteristic component precludes application of a blanket treatment for the Vrica and Stuni specimens. All specimens were subjected to at least two demagnetization temperature steps (350 and 375 °C) and one specimen from each site underwent an additional two to three steps (200, 300 and 400 °C) in order to ensure removal of the present field component without total destruction of the characteristic component.

A site mean and precision parameter was calculated using standard Fisher statistics¹⁰ for the appropriate demagnetization step for each site. Using the test for randomness developed by Watson¹¹, the nonrandom sites were classified as 'A'. The mean direction was then corrected for structural tilt (strike = 0°, dip = 15°W) and converted into a virtual geomagnetic pole (VGP) position. All Class A data, uncorrected for tilt, are plotted on Fig. 2. Class A NRM site means are shown in Fig. 2*a* and cleaned site means are shown in Fig. 2*b*. The mean of the normal sites (declination (dec.) = 22°, inclination (inc.) = 55° with an α_{95} of 5.8°) is antipodal to the mean of the reversed sites (dec. = 213°, inc. = -50° with an α_{95} = 7.5°) at the 95% confidence level and is also significantly different from the present field direction. This finding is considered to be important for the reliable identification of normal polarity zones. In the strongly-remagnetized Vrica and Stuni sediments the test proves to be especially valuable, given the danger of misinterpreting normal polarity zones that have been magnetized in the present field direction as representing an original normal remanence. Based on the positive reversals test and the difference between the mean remanent directions and that of the applied field, it is considered that the magnetozones presented here reflect the Earth's magnetic field at or near the time of deposition of the sediments.

Fig. 2 *a*, Stereonet projection of NRM directions of Stuni and Vrica specimens. Solid symbols indicate lower hemisphere projection, open indicate upper hemisphere. *b*, Projection of statistically significant site means after thermal demagnetization. Symbols are the same as in *a*. *c*, Projection of mean of all significant sites using the antipodes of the reversed sites. The mean is significantly different from the present field direction and the dipole field direction, indicating that the magnetization was acquired before rotation of the site about a vertical axis.



The mean direction of the Stuni and Vrica specimens is significantly different from both the present field and the dipole field at that location, both before and after correction for structural tilt (Fig. 2c). This difference is suggestive of a possible rotation about a vertical axis of about 15° in a clockwise direction, a rotation which is in the opposite sense from the penecontemporaneous rotation of Sicily (J. Besse, personal communication).

Magnetostratigraphy

The VGP latitudes of all class A data are plotted in Fig. 3. Figure 3a shows the Vrica section and Fig. 3b the Stuni. The polarity of these sites is indicated. The polarity interpretation to the right of Fig. 3 is based on the sign of the VGP latitudes of the Class A data (positive being normal and negative being reversed). The polarity zones shown in Fig. 3 have been given informal names (Vrica R1–R4 and N1–N3). Zone N2 should be viewed with caution as it is based on one site only. The Stuni section is separated from the Vrica section by a short (50 m?) gap. For this reason, we have named the reversed polarity zone associated with it R1 and the reversed zone at the base of the Vrica section R2, although we have no normal data between the two.

Note that the normal zones do not represent a recent overprint because the directions are antiparallel to the reversed directions and the mean is significantly different than the present field direction. The mean of the normal sites can be broken into a mean of N1 sites and a mean of N3 sites. The mean of the six statistically significant N1 sites is: dec. = 5.4°, inc. = 57.3°, α_{95} = 3.9°. The mean of the three statistically significant N3 sites is: dec. = 26.3°, inc. = 55°, α_{95} = 28°. The α_{95} of N1 does not include the present field direction and is therefore different at the 95% confidence level. The mean of N3, though well displaced from the present field, has an α_{95} large enough (owing to the small number of sites) to graze the present and dipole field directions. However, the mean of the N3 sites is

significantly different from the present and dipole field directions at the 90% confidence level. The stability of the N3 polarity zone is further illustrated by the thermal demagnetization of specimen VA10A (Fig. 1c) which suggests that the normal zone is stable to at least 400 °C and therefore cannot be of viscous origin⁹.

The results presented here are different from those presented by Nakagawa and Niitsuma¹² who reported additional horizons of normal polarity in the Stuni and Vrica sections. Since their treatment involved a.f. demagnetization to 16 mT followed by thermal treatment to 200 °C, we feel that it is possible that they isolated a present field component, which we found to be very stable against a.f. demagnetization. The a.f. demagnetization tends to destroy the primary remanence and step-wise thermal demagnetization is required to isolate it. It is therefore likely that the additional normal zones present in the stratigraphy of Nakagawa and Niitsuma¹² are the result of an incompletely removed secondary overprint in the present field direction. As these authors have not published the magnetic direction data, it is impossible to define the reasons for the discrepancy between the two data sets.

Discussion

The correlation of the magnetostratigraphy to the geomagnetic reversal time scale (GRTS) is not entirely straightforward owing to the short time span of the sampled section. The radiometric age of 2.2 Myr for the Pliocene ash in the reversed Stuni section⁶ immediately constrains all the overlying strata to be younger than the Gauss–Matuyama boundary (2.47 Myr)¹³. As a result, there are at least three possible correlations of the observed magnetostratigraphy. These are as follows:

- (1) N1–N2 are the Reunion sub-chrons and N3 is the Olduvai.
- (2) N1–N2 comprise the Olduvai and N3 is a short normal subchron between the Olduvai and the Jaramillo.
- (3) N1–N2 comprise the Olduvai subchron and N3 is the Jaramillo.

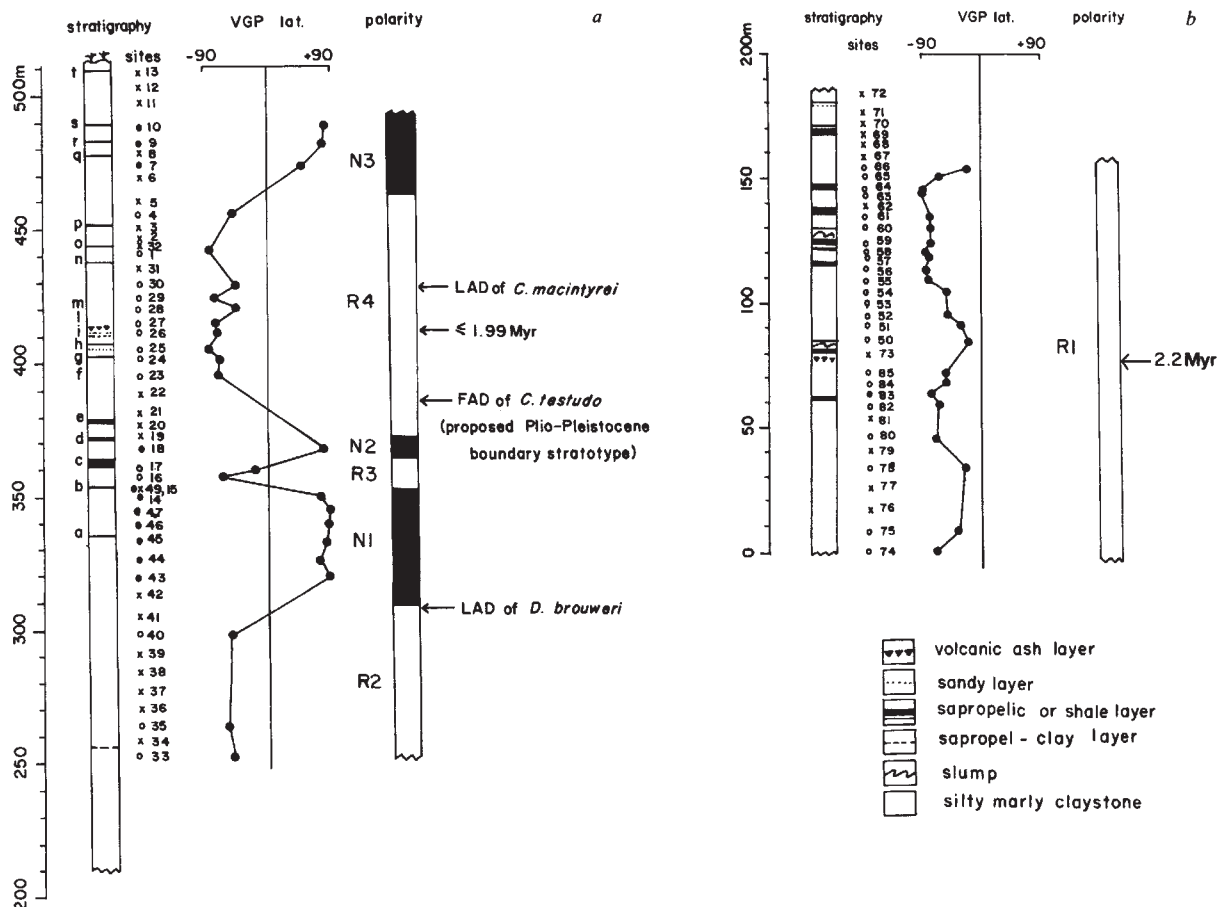


Fig. 3 The magnetostratigraphy of the Vrica and Stuni sections. The Stuni section is located ~1.5 km east of the Vrica section. The lithostratigraphy of the Vrica section is that of Selli *et al.*⁴ and that of the Stuni section was measured by two of us (C.E. and G.P.). The sampling site symbols indicate the polarity of the site: ●, normal; ○, reversed. × indicates that the site did not meet minimum requirements of agreement among specimens from the site. The VGP lat. are calculated from Class 'A' sites. The radiometric dates from ref. 6 are plotted in their stratigraphical positions. The polarity interpretation is shown to the right, black is normal and white is reversed.

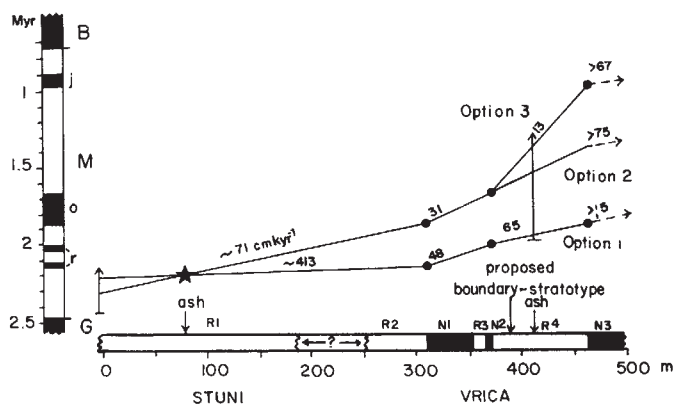


Fig. 4 Age versus stratigraphical position of the three possible correlations (see text). ★, The radiometric date for the lower ash of Obradovich *et al.*⁶. The geomagnetic reversal time scale is that of Mankinen and Dalrymple¹³. B, M and G chrons: B, Brunhes; M, Matuyama; G, Gauss. Subchrons: j, Jaramillo; o, Olduvai; r, Réunion.

Additional evidence which bears on the issue includes the palaeontological evidence of Cati and Borsetti¹⁴, Raffi and Rio (in preparation) and Backman *et al.*¹⁵. The relevant data, including the radiometric ages of Obradovich *et al.*⁶ and the palaeontological data of Backman *et al.*¹⁵ are shown in Fig. 3 to the far right. Note that Obradovich *et al.*⁶ expressed considerable reservation over the age obtained on the 'm' level ash, stressing

that the age of 1.99 Myr is a maximum age for this level and this reservation is acknowledged here. The LAD of *Discoaster brouweri* and *D. brouweri* var. *triradiatus* coincides with the base of the Olduvai normal subchron and that of *Calcidiscus macintyre* occurs above the Olduvai normal subchron in cores from the open ocean¹⁵. These data are recognized at Vrica and occur at the base of N1 and above N2 (Fig. 3 and ref. 15) respectively, suggesting strongly that the N1–N2 normal interval is the Olduvai normal subchron. This seems to indicate that the Réunion subchrons have not been recorded, or are in the 50-m interval that is missing between the Stuni and Vrica sections. From the foregoing discussion, option (1) can be removed from further consideration. Backman *et al.*¹⁵ and Raffi and Rio point out that the continued existence of *Helicosphaera sellii* into the N3 normal polarity zone suggests that N3 is not the Jaramillo (option (3)), but some normal subchron intermediate between the Jaramillo and the Olduvai, although Backman *et al.*¹⁵ note the possibility of reworking. In summary, biochronological criteria favour option (2).

Figure 4 is a plot of stratigraphical thickness against age using the GRTS of LaBrecque *et al.*¹⁷ as refined by Mankinen and Dalrymple¹³ for the various correlations. All three options require substantial differences in rates of sedimentation to accommodate the K–Ar age for the ash in the Stuni section⁶. If N3 is a subchron between the Jaramillo and the Olduvai (option (2)), then it must represent a time span shorter than the resolution of models of magnetic polarity field history based on the magnetic anomaly patterns over the sea floor (approximately 40,000 yr)¹⁷. To record a subchron less than 40,000 yr in duration in the thickness shown in Figs 3 and 4 (at least

30 m) requires a minimum sediment accumulation rate of 75 cm kyr^{-1} for N3, a factor of more than 2 higher than the preceding part of the Vrica section. However, a larger terrigenous input was noted in the uppermost part of the section and based on the lithology this option cannot be excluded.

A final decision on the correlation of the Stuni and Vrica magnetostratigraphy to the GRTS must weigh the likelihood that the selected palaeontological events occurred penecontemporaneously in the open ocean and the Mediterranean versus the likelihood of changes in sediment accumulation rate occurring with minor lithological effects. The arguments of Backman *et al.*¹⁵ for the worldwide penecontemporaneity of the LADs of *D. brouweri* and *D. brouweri* var. *triradiatus* appear to be on firm ground, as opposed to the argument for a more constant sediment accumulation rate which has more aesthetic than scientific merit. The preferred correlation is, therefore, option (2), which correlates N1–N2 to the Olduvai and assumes N3

to be a short subchron between the Olduvai and the Jaramillo. A consequence of the correlation adopted here is that the proposed Plio-Pleistocene boundary stratotype defined in the Vrica section by the physical horizon immediately below the first appearance of *C. testudo* is located above the Olduvai subchron at $\sim 1.6 \text{ Myr}$.

Samples were taken during a field workshop organized by the International Geological Correlation Program's project 128 (Late Cenozoic Magnetostratigraphy) and presided over by Professor Raimondo Selli. Additional support was provided by NSF grants EAR 82 12763 and OCE81-19695. The critical comments of G. Kukla, B. M. Clement, M. L. Colalongo, D. Rio, N. J. Shackleton, J. Backman, J. Van Couvering, J. D. Obradovich and E. Hailwood, are greatly appreciated. The palaeomagnetic laboratory was maintained by D. Lafferty and the support of D. Kent is acknowledged. Lamont-Doherty contribution no. 3482.

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Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing

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Alternative processing of the RNA transcribed from the calcitonin gene appears to result in the production of a messenger RNA in neural tissue distinct from that in thyroïdal 'C' cells. The thyroid mRNA encodes a precursor to the hormone calcitonin whereas that in neural tissues generates a novel neuropeptide, referred to as calcitonin gene-related peptide (CGRP). The distribution of CGRP-producing cells and pathways in the brain and other tissues suggests functions for the peptide in nociception, ingestive behaviour and modulation of the autonomic and endocrine systems. The approach described here permits the application of recombinant DNA technology to analyses of complex neurobiological systems in the absence of prior structural or biological information.

THE nervous system is unique among organ systems in the complexity and specificity of its intercellular communications, which consist primarily of short-range interactions at chemical synapses between the chains of neurones that mediate sensory, integrative and motor functions. It seems likely that the unique phenotypic properties of many of the cells in the nervous system are due in part to their ability to synthesize and release, as well as respond to, a wide variety of polypeptide regulators. The peptide neurotransmitters that have been characterized so far are likely to represent only a small percentage of the total number utilized by the nervous system, and have classically been obtained by purification from large quantities of tissue on the basis of a specific bioassay. Once purified, sequenced and

synthesized, study of these known polypeptide regulators using histochemical, physiological and behavioural assays has demonstrated that each polypeptide is localized to a discrete subset of neurones and pathways in the central nervous system. Each polypeptide subserves a wide variety of functions that are, in part, dependent on the site of synthesis. Furthermore, these neuropeptides are also located in tissues outside the brain, such as the gastrointestinal tract or lung, and are known to exert regulatory functions on extraneuronal target tissues.

In this article we describe an approach by which recombinant DNA and molecular biological techniques can be applied to the identification of previously unknown neuropeptides. This approach was applied in the identification of a putative novel

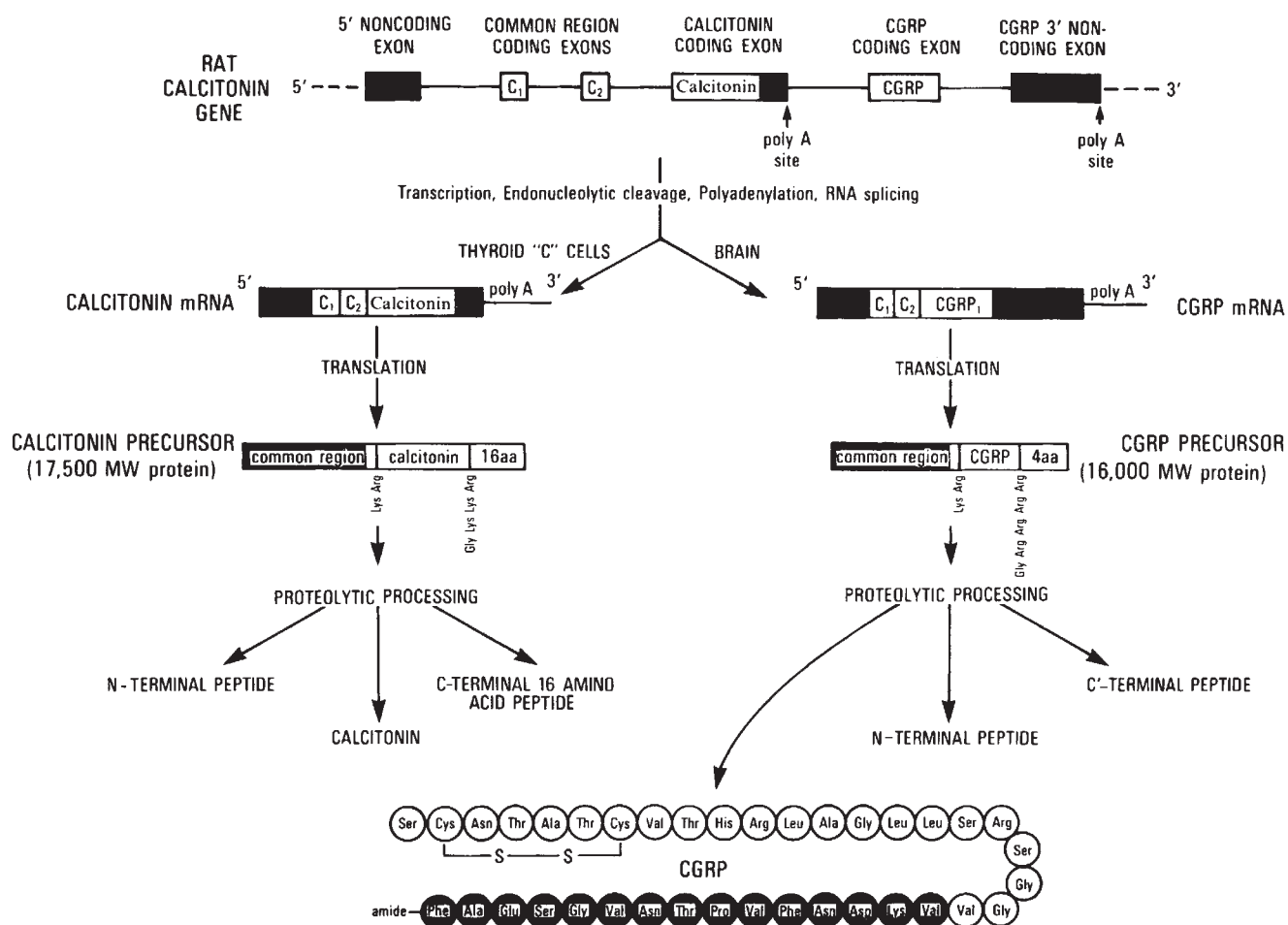


Fig. 1 Alternative RNA processing pathways in expression of the calcitonin gene, which predict the synthesis of a novel neuropeptide (CGRP) in the brain. The structural organization of the rat calcitonin gene is based on DNA sequence and mapping data¹. The selection of alternative polyadenylation sites is suggested to account for the synthesis of calcitonin in mRNA in thyroidal 'C' cells, and the larger CGRP mRNA in brain. CGRP mRNA from rat medullary thyroid carcinomas encodes a 16,000 MW primary translation product, which we predict would be processed to generate three polypeptides, including the putative 37 amino acid polypeptide referred to as CGRP.

Methods: The immunological strategy was analogous to one successfully used to generate site-directed somatostatin antisera¹¹. A synthetic oligopeptide consisting of the predicted ultimate 14 C-terminal amino acids of CGRP (shaded), with an additional N-terminal tyrosine residue to permit linkage and iodination for radioimmunoassay, was assembled automatically using a 990 B Beckman synthesizer, with a previously reported program¹². Hydrogen fluoride cleavage was performed using the standard technique. The crude peptide was purified on an ion-exchange column (CM52) using a gradient of ammonium acetate from 0.01 M (pH 4.5) to 0.3 M (pH 6.5). The major component was subjected to countercurrent distribution after it had been desalted by multiple lyophilizations from distilled water. The solvent system used was 0.1% 1-butanol, and 200 transfers were performed with the main fraction being collected in tubes 19–25. The overall yield was 10% based on the substitution per g of starting resin. Reverse-phase HPLC in several systems indicated that the product had a purity of >95%. Amino acid analysis after 4 M methane sulphonic acid (containing 0.2% tryptamine) hydrolysis gave the predicted ratios. The synthetic peptide was linked by bisdiazotization through the tyrosine to human α -globulins (Sigma). Antisera were raised in New Zealand white rabbits by serial multiple intradermal injections of the conjugate emulsified in Freund's adjuvant. Rabbits were injected every 2 weeks, and sera obtained 10 days after the third and sixth bleedings were adsorbed with excess human α -globulins and used for studies reported here.

neuropeptide, predicted on the basis of structural analysis of calcitonin gene expression¹. Based on the evidence presented below we suggest that, in the brain, the RNA transcribed from the calcitonin gene is processed to a discrete mRNA encoding the precursor to a predicted 37 amino acid peptide, referred to as CGRP (calcitonin gene related peptide).

Alternative RNA processing

We have recently reported how alternative RNA and protein products of the calcitonin gene can be generated in specific tissues by the selection of alternative exons for incorporation into their respective mRNAs^{1–5} (see Fig. 1), rather similar to the generation of multiple mRNAs from a single transcription

unit in viral and immunoglobulin gene expression^{7–10}. Thus, the calcitonin gene encodes two different mRNAs that share an identical 5' sequence, but have entirely different 3' sequences¹. The resultant mRNAs encode either the 17,500 molecular weight (MW) calcitonin precursor protein, which is proteolytically processed to yield the calcium-regulating hormone, calcitonin, and two other peptides^{2–4}; or a 16,000 MW protein, the predicted translation product of CGRP mRNA (see Fig. 1). From the nucleotide sequence of the cloned CGRP cDNA it can be predicted that the encoded protein is proteolytically processed to generate three peptides, including the 37 amino acid CGRP (excised from the precursor by N-terminal cleavage at the dipeptide Lys-Arg, and C-terminal cleavage at the sequence Gly-Arg-Arg-Arg).

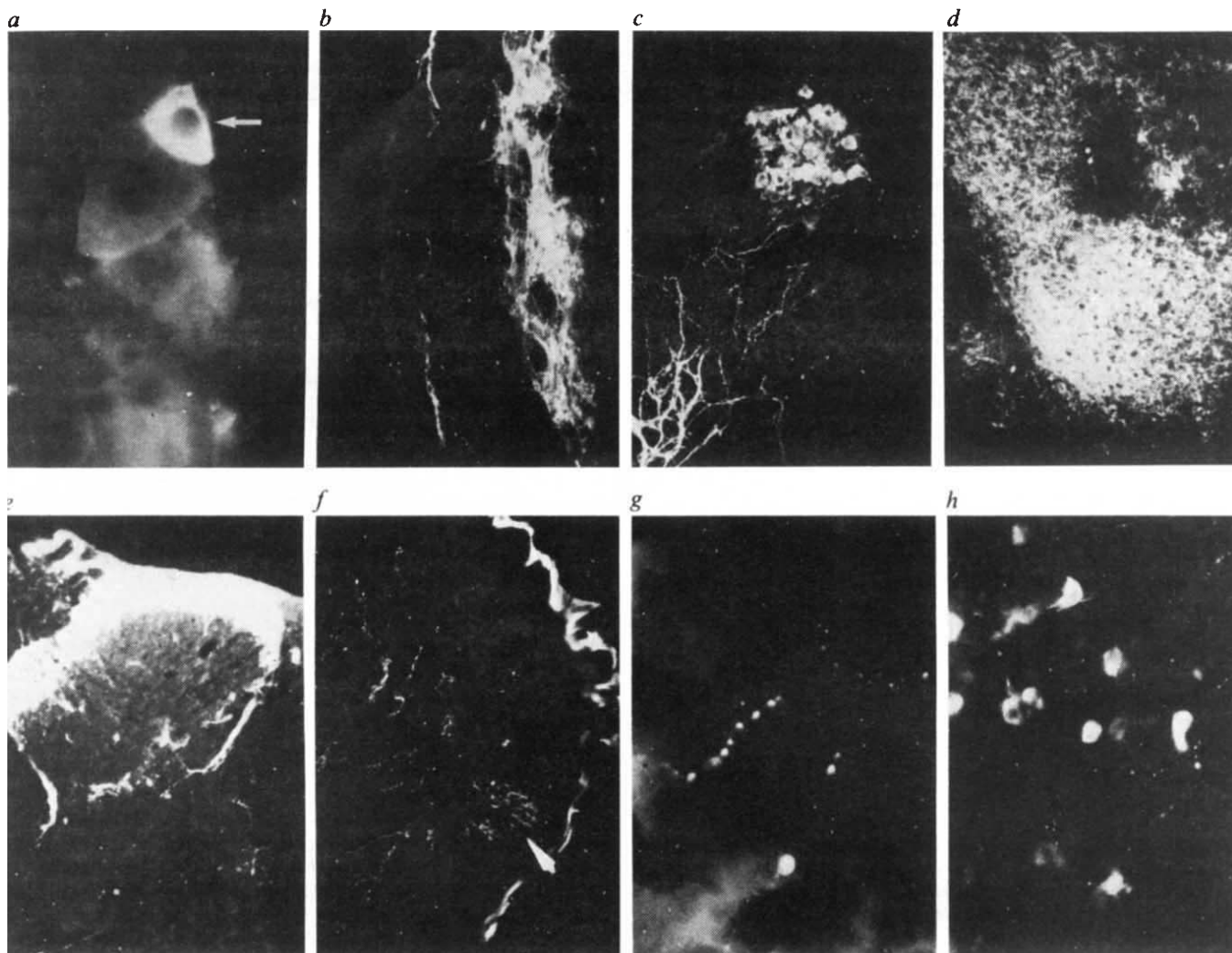


Fig. 2 Immunofluorescence localization of CGRP in the nervous system. *a*, High-power view of a row of four neurones in the trigeminal ganglion. Three large unstained neurones can be seen below one small, intensely stained neurone, the nucleus of which is unstained (arrow) ($\times 500$). Trigeminal ganglia contained 0.25–0.7 ng immunoreactive CGRP per mg tissue. *b*, A dense band of CGRP-stained fibres in the superficial part of the spinal trigeminal nucleus, pars caudalis. The lateral surface of the medulla is to the left. A few stained fibres, which arise in the trigeminal ganglion (*a*), can also be seen in the spinal tract of the trigeminal nerve, which lies just medial to the edge of the brain stem ($\times 75$). *c*, CGRP-stained motorneurons in the rostral part of the nucleus ambiguus (top). Stained fibres associated with the trigeminal input to the lateral medulla can be seen in the lower lateral field to the left ($\times 75$). *d*, A dense circumscribed CGRP-stained terminal field in the lateral part of the central nucleus of the amygdala. Fibres associated with the terminal field also formed patchy terminal fields in caudal parts of the caudoputamen and globus pallidus ($\times 75$). *e*, CGRP-immunoreactive fibres in the dorsal horn (substantia gelatinosa) of the spinal cord at segment T₁₀. Because many stained fibres were seen in dorsal roots, it seems clear that the fibres shown here arise from dorsal root ganglion cells, as in the trigeminal nucleus. In each section of the spinal cord, several large motor neurones in the ventral horn were labelled, although stained preganglionic sympathetic neurones in the intermediolateral column were not observed ($\times 75$). *f*, A cross-section through the tongue, the lateral edge of which is outlined by scalloped nonspecific staining on the right. Many CGRP-stained fibres were seen among muscle fibres and within the walls of blood vessels, as well as within some, but not all, taste buds (arrow) ($\times 75$). *g*, CGRP-stained fibres in the anterior lobe of the pituitary gland ($\times 500$). *h*, CGRP-stained cells and fibres in the adrenal medulla ($\times 200$).

Methods: Immunofluorescence localization of CGRP was carried out as described in detail in ref. 13. Serum was treated by addition of human α -globulin ($7.5 \mu\text{g ml}^{-1}$). Staining was completely blocked by the addition of synthetic CGRP (23–37) or CGRP (1 mg); addition of calcitonin, vasopressin or somatostatin (1–5 mg) did not influence staining. 24 Hours before perfusion, animals were treated either with $75 \mu\text{g}$ of colchicine subcutaneously (*a*, *f–h*) or $150 \mu\text{g}$ of colchicine in the lateral ventricle (*b–d*, *e*). Radioimmunoassay was used to quantitate CGRP content in trigeminal ganglia. Serum was used at a dilution of 1:85,000, with assay sensitivity to 0.5 pg. Competition by intact CGRP was about 30% as effective as CGRP (23–37); CRF, GRF, calcitonin and somatostatin failed to compete with immunoprecipitation of iodinated CGRP (23–37).

The specific hybridization of CGRP exon-specific probes to poly(A)-selected RNA from rat trigeminal ganglia and hypothalamic RNA preparations, but not from thyroidal 'C' cells (data not shown), suggested that the critical physiological consequence of these alternative RNA processing pathways might be the tissue-specific synthesis of CGRP in the brain (see Fig. 1). The results of immunohistochemical studies, peptide isolation and characterization, and RNA structural analyses, support the prediction that CGRP is produced in the central and peripheral nervous systems.

Localization of CGRP immunoreactivity

Antisera raised against a synthetic polypeptide corresponding to a portion of the predicted C-terminal sequence of CGRP (Fig. 1) were used for immunohistochemical studies of the rat. Photomicrographs of representative areas are shown in Fig. 2. Specific immunostaining was observed in restricted components of neuronal systems that are known to subserve sensory, integrative and motor functions (see Fig. 3). Staining in sensory systems includes a subpopulation of small neurones in the

trigeminal ganglion (Fig. 2a) and in the spinal sensory ganglia, as well as their terminals in the brain stem and spinal cord, which are believed to relay somatic and cutaneous pain and/or temperature information. The overall distribution of the CGRP-stained neurones and pathways does not correspond to that of any other known neuropeptide. The CGRP content of trigeminal ganglia, as quantitated by radioimmunoassay, is 0.3–0.7 ng per mg tissue (Fig. 2 legend). There are also many CGRP-stained fibres in the tongue, some of which innervate a subset of taste buds (Fig. 2f), and a subset of primary olfactory fibres are CGRP immunoreactive. Most of the motor neurones in the facial nucleus, the hypoglossal nucleus (tongue movement) and in rostral parts of the nucleus ambiguus (innervation of the heart and the branchial muscles of the pharynx and larynx that are involved, for example, in swallowing) (Fig. 2c) contain immunoreactive CGRP, which suggests that this neuropeptide is produced in neurones that use acetylcholine as a neurotransmitter.

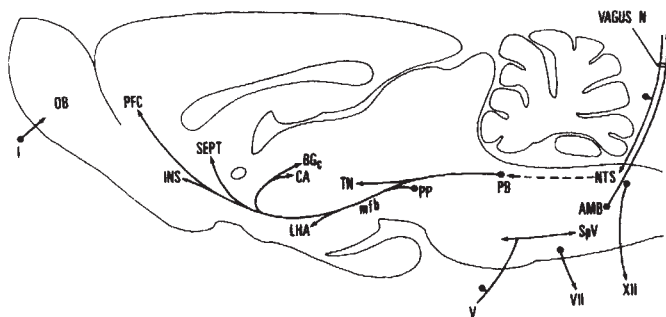


Fig. 3 A summary of the major CGRP-stained cell groups (black dots) and pathways (arrows) projected on a sagittal view of the rat brain. This staining was localized in discrete parts of several functional systems. First, dense terminal fields were stained throughout the substantia gelatinosa of the spinal cord and caudal part of the spinal trigeminal nucleus. These fibres arise in dorsal root and trigeminal ganglion cells (Fig. 2a) and probably relay nociceptive or thermal information. Second, CGRP is found in most parts of the taste pathways, including sensory endings in taste buds and the central endings of these fibres in the rostral part of the nucleus of the solitary tract (NTS), and in the relay system from the parabrachial nucleus (PB) to the thalamic taste nucleus (TN) and the taste area of the cerebral cortex (posterior agranular insular area, INS). In addition, most motor neurones in the hypoglossal nucleus (XII), which move the tongue, were stained. Third, a small group of primary olfactory fibres (I) that end in the glomerular layer of the olfactory bulb (OB) were stained, suggesting that CGRP has a role in olfaction as well as taste. Fourth, a small number of fibres, apparently from the 8th nerve, end in the cochlear and vestibular nuclei (not shown). Fifth, CGRP is found throughout the caudal part of the NTS, and throughout the PB, suggesting that it plays a part in the relay of visceral sensory information from the vagus (and glossopharyngeal) nerve, by way of an ascending pathway through the medial forebrain bundle (MFB). This pathway appears to arise in the PB and peripeduncular nucleus (PP), and projects to the lateral hypothalamic area (LHA), the central nucleus of the amygdala (CA), to patches in caudal parts of the caudoputamen and globus pallidus (BCc), to the lateral septal nucleus and bed nucleus of the stria terminalis (SEPT) and to layer III of three cortical areas: the infralimbic prefrontal area (PFC), the INS and the perirhinal area. The ascending projections in the MFB are probably modulated by a massive, non-CGRP containing pathway from the NTS to the PB (dashed line). Sixth, stained motor neurones in the rostral part of the nucleus ambiguus (AMB) project through the vagus nerve and may innervate the heart and/or branchial muscles in the pharynx that are involved, for example, in the control of swallowing. The overall distribution of CGRP-stained pathways does not correspond with any other known peptide, and suggests that it has a particularly important role in the sensory, integrative (by way of the MFB) and motor components of ingestive behaviour, and also in the processing of painful stimuli.

CGRP-staining was also observed in thin, beaded fibres throughout many parts of the body, including the heart, lung and gastrointestinal tract, and these fibres were often associated with the smooth muscle of blood vessels. The thyroid gland, despite the fact that it does receive sensory, sympathetic and parasympathetic innervation, failed to demonstrate CGRP-staining in either cells or fibres. Conversely, antisera against calcitonin failed to stain any region of the brain or sensory ganglia, although bright 'C' cells and beaded fibres were observed in the thyroid.

The possibility that CGRP has a significant role in the endocrine system is suggested by the presence of a subset of cells that are stained by the CGRP antisera in the adrenal medulla (Fig. 2h), and by CGRP-stained fibres that project through the adrenal cortex to a subcapsular zone, where they are particularly numerous. A small number of CGRP-stained cells and fibres were also observed at the periphery of pancreatic islets. While no CGRP-stained cells were observed in the small bowel of the rat, occasional cells did stain with the calcitonin antisera as did a few cells in the centre of some pancreatic islets. Scattered CGRP-stained beaded fibres were observed in the anterior lobe of the pituitary gland (Fig. 2g). These fibres could not be traced from the brain, and may represent 'sympathetic' fibres arising in the superior cervical ganglion.

CGRP mRNA in brain

It is important to demonstrate that it really is CGRP that is being recognized by the antisera in the immunohistochemical experiments, and not just cross-reacting, structurally related antigens. The recent identification of a second gene encoding a CGRP-related peptide (our unpublished data) makes this question particularly pertinent. Evidence that the CGRP staining does represent calcitonin gene expression has been obtained by using S_1 nuclease mapping experiments¹⁴ to detect the presence and distribution of authentic CGRP mRNA in the brain.

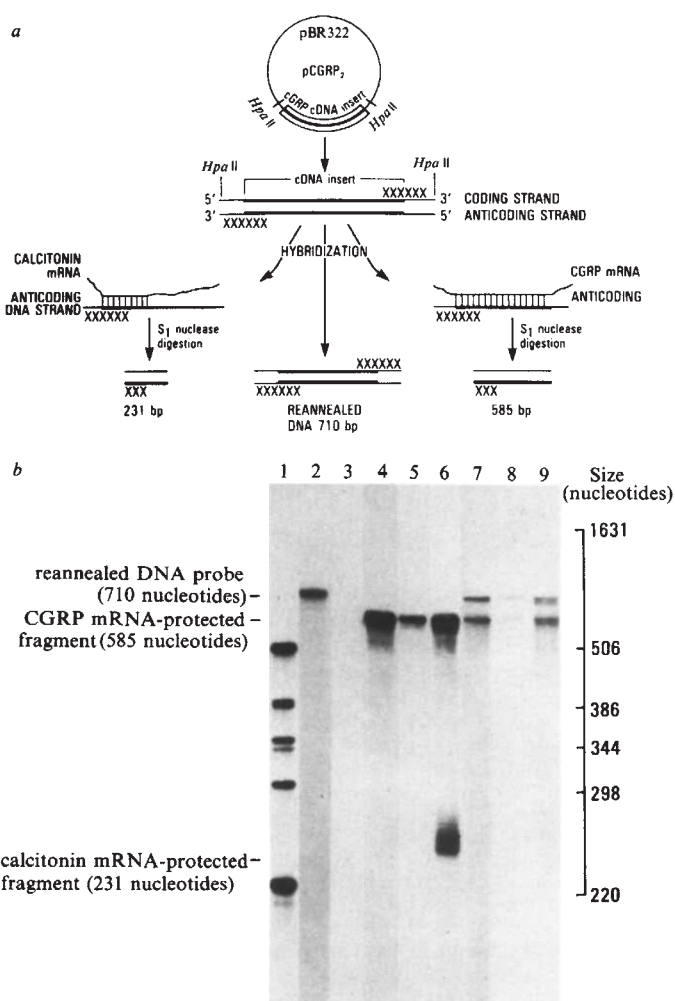
The experimental approach used is shown schematically in Fig. 4a. The cDNA insert of plasmid pCGRP₂ is complementary to the 5' sequence shared by both calcitonin and CGRP mRNAs, and also to the 3' sequence unique to CGRP mRNA, so S_1 nuclease protection assays simultaneously quantitate the amount of both calcitonin and CGRP mRNAs. CGRP mRNA is distinguished in this analysis from the product of the second gene encoding a CGRP-related peptide, the mRNA of which has a primary structure that differs considerably from that of CGRP mRNA. The results of such an analysis are shown in Fig. 4b. CGRP mRNA was identified in RNA prepared from the hypothalamus (medial preoptic area cells), midbrain (peripeduncular nucleus cells), and trigeminal ganglion. RNA prepared from a region containing primarily terminal fields (amygdala) contained only very low levels of CGRP mRNA. Calcitonin RNA sequences were not detected in any of the brain regions analysed, but were readily detected in a rat medullary thyroid cell line¹⁵ that simultaneously produces calcitonin and CGRP mRNAs. This analysis confirms that authentic CGRP

Table 1 Quantitative analysis of CGRP mRNA in the brain

Region assayed	CGRP mRNA	Calcitonin mRNA
Trigeminal ganglion	100	ND
Lateral medulla	4.8	ND
Midbrain	0.4	ND
Hypothalamus	0.4	ND
Amygdala	<0.1	ND

Quantitative densitometric scan analysis of autoradiograms similar to those shown in Fig. 4b, with adjustments for the time of exposure such that each band analysed was within the linear portion of the exposure. Results are presented as per cent of the maximal protection (observed using trigeminal nuclear poly(A)-rich RNA). The medullary thyroid cell line RNA standard is from a clonal line of rat medullary thyroid cells which produce extremely low levels of both calcitonin and CGRP mRNAs. ND, None detectable.

Fig. 4 S_1 nuclease mapping of CGRP mRNA sequences in the brain. **a**, The strategy used for S_1 nuclease analysis. The plasmid pCGRP₂, which contains a 585 bp sequence of CGRP cDNA inserted into the *Pst*I site of pBR322, was restricted with *Hpa*II such that an excised 710 bp fragment contains the 585 bp CGRP cDNA insert flanked by short sequences contributed by pBR322. This fragment provided a convenient probe for analysis of CGRP mRNA. The fragment was labelled by first incubating in 33 mM Tris-Ac (pH 7.9), 66 mM KAc, 10 mM MgAc, 100 μ g ml⁻¹ bovine serum albumin, 5.4 mM dithiothreitol for 20 min at 37 °C with 0.4 U ml⁻¹ T4 DNA polymerase to permit 3' exonuclease digestion, and then adding deoxyribonucleotides, including 100 μ Ci of [α -³²P]dCTP (300 Ci mmol⁻¹), and continuing to incubate for 10 min such that the digested DNA is quantitatively replaced (2 mM unlabelled dCTP was added to 0.1 mM final concentration during the final minute of the reaction). Following phenol/chloroform extraction and ethanol precipitation, the labelled *Hpa*II fragment was subjected to electrophoresis through a 5% polyacrylamide gel. The DNA was eluted, melted and aliquots (50,000 c.p.m., 5×10^7 – 5×10^8 c.p.m. per μ g DNA) were mixed with buffer containing 20 μ g of poly(A)-selected RNA from the tissue being analysed in the presence of 10 μ g *Escherichia coli* tRNA and ethanol precipitated. The nucleic acids were dissolved in 40 μ l of hybridization buffer containing 50 mM PIPES (pH 6.4), 500 mM NaCl, 1 mM EDTA, 80% formamide, heated to 85 °C for 10 min, then placed at 55 °C for 3 h. The reaction was diluted 10-fold with buffer containing 280 mM NaCl, 30 mM NaAc (pH 4.5), 4.5 mM ZnSO₄ and 20 μ g ml⁻¹ single-stranded DNA and incubated for 30 min at 37 °C with 500 U of S_1 nuclease. Following the nuclease digestion, the sample was subjected to phenol extraction, ethanol precipitated, resuspended in 5 μ l of formamide and subjected to electrophoresis using a 4% acrylamide, urea gel. Any reannealed DNA will be 710 bases in length; 585 nucleotides of radiolabelled fragment will be protected from S_1 nuclease-digestion if hybridized to CGRP mRNA, while 231 nucleotides of radiolabelled fragment will be protected from S_1 nuclease digestion if hybridized to calcitonin mRNA. In the absence of added RNA, only the trace amount of reannealed DNA would remain undigested. **b**, Analysis of S_1 nuclease protection of CGRP cDNA using brain poly(A)-rich RNA. Autoradiographs of: lane 1, *Hinf*-digested pBR322 standards (3 h exposure); lane 2, aliquots of undigested probe (21 h exposure); lane 3, probe sham hybridized with carrier RNA only (72 h exposure); lane 4, hybridization to 20 μ g poly(A)-rich RNA from trigeminal ganglia (3 h exposure); lane 5, hybridization to 20 μ g poly(A)-rich RNA lateral medulla (11 h exposure); lane 6, hybridization to 17 μ g poly(A)-rich RNA from the clonal rat medullary thyroid cell line (ref. 19, a gift of F. Zeytinoglu) (11 h exposure); lane 7, hybridization to 20 μ g poly(A)-rich RNA from midbrain (72 h exposure); lane 8, hybridization to 20 μ g poly(A)-rich RNA from temporal tissue (amygdala) (72 h exposure); lane 9, hybridization to 20 μ g poly(A)-rich RNA from hypothalamus (72 h exposure). The migration of reannealed, CGRP mRNA-protected and calcitonin mRNA-protected probe are indicated. Similar results were obtained in three independent analyses.



mRNA is produced in regions of the central nervous system that contain CGRP-immunoreactive cell bodies.

CGRP mRNA levels were quantitated by a densitometric scan analysis of autoradiographs similar to that shown in Fig. 4b, and the results of such an analysis are shown in Table 1. The levels of CGRP mRNA are considerably higher in the trigeminal ganglion than in specific regions of the brain. CGRP RNA sequences are approximately 0.5–1% as frequent in the trigeminal ganglion as in rat medullary thyroid tumours, which contain ~10,000 copies per cell. On the basis of immunohistochemical reactivity, it was estimated that about 1% of trigeminal ganglia neurones¹⁶ are CGRP-producing, and that they contain ~4,000–20,000 copies of CGRP mRNA per cell (similar to the levels of prolactin and growth hormone mRNAs in their respective cells of origin in the anterior pituitary¹⁷).

The rate of transcription of the calcitonin gene in the trigeminal ganglion was estimated by DNA-excess hybridization experiments, after labelling the nascent RNA transcripts in nuclei isolated from trigeminal ganglia by incubation with [α -³²P]UTP as described previously^{17,18}, as 200–300 p.p.m. per kilobase (kb) of DNA probe in each CGRP-synthesizing neurone (which compares well with a rate of 100–300 p.p.m. per kb probe estimated for medullary thyroid calcitonin-producing tumours).

The relative level of CGRP mRNA in each brain region parallels the percentage of CGRP-reactive neurones present in

each region assayed (see Table 1). In an area rich in CGRP-stained terminal fields (central nucleus of the amygdala), very little CGRP mRNA was detected. No calcitonin mRNA sequences were detected in any brain region, even though the S_1 nuclease assay used would easily detect transcripts present at concentrations of less than 0.2% of that of CGRP mRNA. Thus, if any calcitonin-synthesizing neurones are present in the brain, they would have to represent a very small number of cells producing extremely small quantities of calcitonin mRNA and immunoreactive calcitonin to escape detection.

Protein encoded by CGRP mRNA

From the sequence of CGRP mRNA it is predicted that it encodes of 16,000 MW protein, the precursor of CGRP. As shown in Fig. 5, hybridization of rat trigeminal ganglia poly(A)⁺ RNA to an immobilized plasmid containing a CGRP-specific insert specifically selects a mRNA that directs the synthesis of a 16,000 MW protein in a cell-free translation system. This product co-migrates with the protein encoded by the authentic CGRP mRNA isolated from rat medullary tumour cells. mRNA from the lateral medulla (presumably including the nucleus ambiguus and facial nucleus) also directed the synthesis of the 16,000 MW protein. Thus, brain CGRP mRNA sequences appear to direct the synthesis of the protein precursor predicted by the CGRP cDNA sequence.

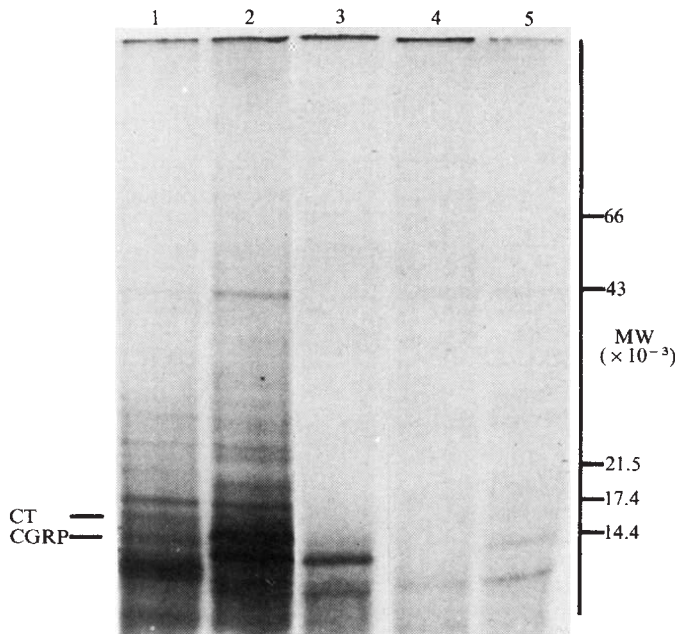


Fig. 5 Cell-free translation of CGRP mRNA from the brain. Autoradiograms demonstrate the translation products directed by: lane 1, poly(A)-rich RNA from trigeminal ganglia; lane 2, poly(A)-rich RNA from a medullary tumour; lane 3, medullary tumour RNA selected by the CGRP-specific plasmid; lane 4, trigeminal ganglion RNA selected by pBR322 as a control; lane 5, trigeminal ganglion RNA selected by the CGRP-specific plasmid. The migration of calcitonin and CGRP precursors is indicated. The ^{35}S -methionine-labelled bands observed in the pBR322 control lane are present in the absence of any added RNA and are thought to represent the translation products of endogenous wheat germ mRNAs.

Methods: Poly(A)-rich RNA prepared from trigeminal ganglia (5 μg) or from a rat medullary tumour producing both calcitonin and CGRP mRNAs (1.6 μg) was allowed to hybridize to plasmids immobilized on nitrocellulose. The hybridization selection and elution were performed as described previously¹⁹ using nitrocellulose filters to which 2 μg of denatured, linearized plasmid containing a CGRP mRNA-specific cDNA insert (lanes 3, 5) or pBR322 (lane 4) had been bound. The RNAs eluted from both control (pBR322) and CGRP-specific filters were translated in a nuclease-treated wheat germ lysate, and the ^{35}S -methionine-labelled products were subjected to SDS-polyacrylamide gel electrophoresis, as described previously².

However, the presence of CGRP mRNA and CGRP antisera immunoreactivity in brain cells does not conclusively establish the protein-processing scheme shown in Fig. 1. Evidence that CGRP itself really is the product of calcitonin gene expression in the brain was obtained by characterization of CGRP-immunoreactive material derived from rat hypothalami. The predominant component of the immunoreactive material is a small peptide, estimated by gel filtration to have a MW of 4,200 (Fig. 6), and which co-migrates with synthetic CGRP standard on HPLC (unpublished data). These data strongly support the scheme shown in Fig. 1 for the generation of CGRP in the brain.

Concluding remarks

An analysis of calcitonin gene expression has provided the first example of tissue-specific regulation of RNA processing pathways in the nervous and endocrine systems, which may be one of the ways that diversity in the neuroendocrine system is increased and which may have important physiological consequences. Calcitonin gene expression, therefore, provides a useful model system for exploring the mechanisms responsible for developmentally determined RNA processing, and for defining an approach to investigate the production, distribution and function of neuropeptides predicted to be made by nucleotide sequencing. This approach is based on the use of antisera against a synthetic polypeptide (representing part of the

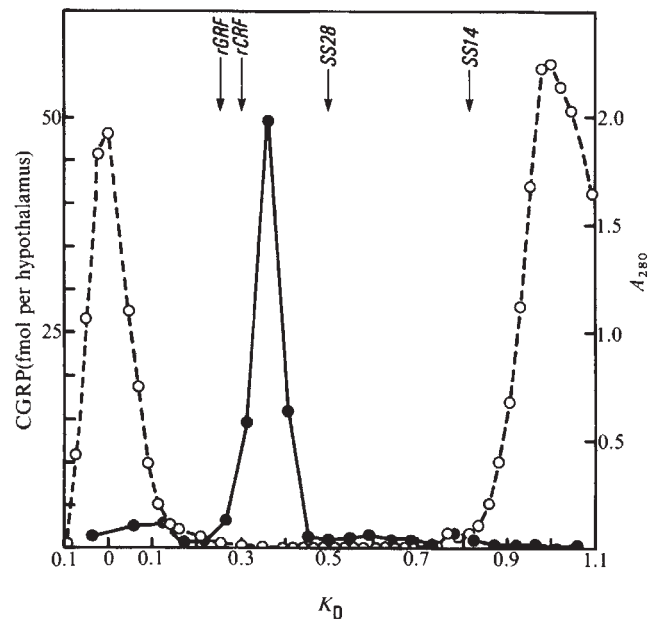


Fig. 6 Gel filtration of a defatted acetic acid extract of 20,000 rat hypothalamic fragments. Extraction and gel filtration (G50 fine) were performed as described previously²⁰ using a Pharmacia K 15/100 column (21.5 $\times$ 90 cm, V_t = 32.6 L). The column was eluted with 3 M acetic acid, 0.1% β -mercaptoethanol at a flow rate of 750 ml h^{-1} . Each fraction was quantitated for material immunoreactive to CGRP antiserum using radioimmunoassay. Elution of rat growth hormone releasing factor (rGRF), rat corticotropin-releasing factor (rCRF), somatostatin (●—●), 14 (SS14), and somatostatin-28 (SS28) are indicated by arrows. $K_D = V_e - V_0 / V_t - V_0$; where V_e = elution volume; V_0 = void volume; V_t = total volume. A semi-logarithmic plot of molecular weight standards suggests a theoretical molecular weight of the CGRP-immunoreactive material of 4225.

encoded protein) and S_1 nuclease mapping, which permit simultaneous analysis of both the synthetic pathways involving the peptide and the neuronal sites of peptide synthesis. The modified S_1 nuclease protection assay used here confirms that CGRP mRNA is made in the brain and identifies the sites of its biosynthesis. CGRP mRNA in the brain is regionally distributed in a way that corresponds to the immunohistochemical distribution of the reactive peptide, and brain CGRP mRNA directs the cell-free synthesis of the predicted 16,000 MW CGRP precursor protein. That this precursor really is processed by proteolysis in the brain is demonstrated by the size of the brain immunoreactive CGRP; unequivocal evidence that no other post-translational modifications occur can be obtained only by sequencing the purified peptide.

Immunoreactive calcitonin and calcitonin mRNA were not detected in rat brain or pituitary. Earlier reports of immunoreactive calcitonin in the pituitary gland and/or brain of the rat were based on immunohistochemical or radioimmunoassay evidence²¹⁻²⁵. On the basis of the data presented here, and of RNA blot analyses of the pituitary^{2,25}, the immunological data in rats probably reflect either the detection of a substance cross-reacting with calcitonin antisera, or the binding of calcitonin from the blood that has entered circumventricular regions of the brain that lack a blood-brain barrier to peptides. Calcitonin membrane receptor sites in the hypothalamus and pituitary of rat and human brain have been reported^{26,27}, although it is possible that these receptors bind a calcitonin-related peptide rather than calcitonin itself. Reports of immunoreactive calcitonin in the central nervous system of birds²⁸, reptiles²⁹, protochordates³⁰ and sea squirts³¹ could reflect either a different organization of the calcitonin gene, altered developmental switching events, or binding of calcitonin from the blood. With regard to these possibilities, we find that the chicken hypothalamus contains large numbers of CGRP-

stained cells and fibres, consistent with the presence and expression of CGRP exons in the chicken calcitonin gene.

The distribution of CGRP in specific sensory, integrative and motor systems suggests several possible functions for the peptide. The differential distribution of CGRP immunoreactivity in the olfactory and gustatory systems, in the hypoglossal, facial and vagal nuclei, and in the hypothalamus and limbic regions, strongly suggests that it may have a functional role in ingestive behaviour. CGRP is present in small trigeminal and spinal sensory ganglion cells, which are known to relay thermal and nociceptive information from the head and body to the brain stem and spinal cord. Applying a method combining immunohistochemical and fluorescence retrograde transport techniques³², CGRP was also shown to be present in many of the motor neurones in rostral, but not caudal, parts of the nucleus ambiguus (Fig. 2). This part of the nucleus ambiguus is believed to be particularly concerned with certain visceral motor functions mediated by the vagus nerve. The production

of a polypeptide hormone by cholinergic cells has already been suggested for vasoactive intestinal peptide (VIP)³³. CGRP is also present and widely distributed in fibres in blood vessels and the visceral organs, and in the adrenal medulla where, as in the sensory ganglia, CGRP is one of several neuropeptides (for example VIP, substance P, enkephalins), each present in a subpopulation of cells^{34,35}. This distribution suggests CGRP may be involved in cardiovascular regulation. In fact, administration of synthetic CGRP exerts profound and unique effects on blood pressure and blood catecholamine levels in the rat (L. Fisher *et al.*, manuscript submitted). Finally, the production of CGRP in several glandular tissues suggests that it may also have endocrine functions.

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Translocation joins *c-myc* and immunoglobulin $\gamma 1$ genes in a Burkitt lymphoma revealing a third exon in the *c-myc* oncogene

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Recombinant DNA clones have been used to directly demonstrate that the Burkitt lymphoma cell line Raji, t(8;14)(q24;q32), has a rearranged copy of the c-myc gene adjacent to the $\gamma 1$ constant region gene of the human immunoglobulin heavy-chain locus; the genes are arranged in the opposite direction for transcription. At least one further 5' exon has been detected in the normal c-myc gene by analysis of RNA transcripts, and the occurrence of high levels of two c-myc mRNA size classes, both apparently initiating in this exon, is described.

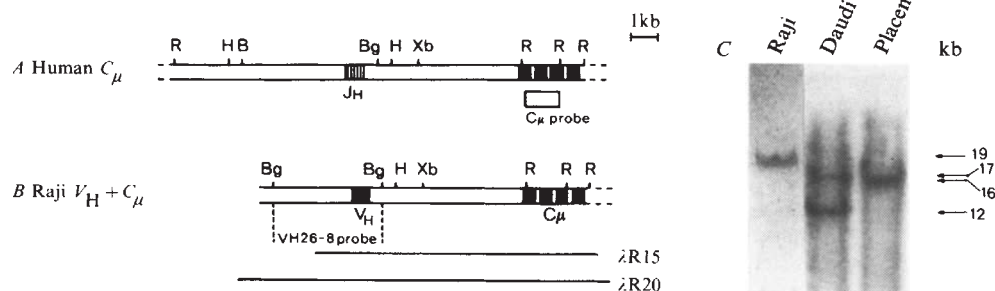
BURKITT'S lymphoma (BL) is a form of B-cell leukaemia that occurs in man. An interesting feature of these tumours is that the cells almost invariably carry specific chromosomal translocations involving a segment of chromosome 8(8q24→qter)¹. This translocation frequently involves reciprocal exchange with the long arm of chromosome 14 (ref. 1) at q32. Variant translocations of chromosome 8 with chromosome 2(2p12) or 22(22q11) are also known². Recent genetic mapping studies have shown that the human heavy(H)-chain genes are located on chromosome 14 (refs 3, 4), the κ light(L) chains on chromosome 2 (ref. 5) and the λ light chains on chromosome 22 (ref. 6): furthermore, the localizations have been refined to 14q32, 2p12 and 22q11 for H, κ and λ chains respectively^{5,7,8}. Thus,

it was strongly suggested that the immunoglobulin genes are directly involved in these specific translocations in BL.

A gene sequence from chromosome 8 which is implicated in the BL translocations is the human homologue of the avian myelocytomatosis virus oncogene, designated *c-myc*. This gene has been mapped to the position on human chromosome 8 which is involved in the BL translocation (8q24)^{9,10}, and indeed Southern filter hybridization indicates that the *c-myc* and the μ H-chain constant(C) region gene are present, in a small proportion of BL cells, on the same restriction fragment^{11,12}.

We now present direct proof that the *c-myc* gene is translocated into the H-chain locus in a BL cell line (Raji) by cloning, in λ phage, the rearranged and non-rearranged *c-myc* genes

Fig. 1 Organization of the C_μ gene in Raji cell DNA. **A**, Genomic map of C_μ gene region indicating the C_μ probe. **B**, Total DNA from Raji cells (t8;14) was partially digested with *MboI* and ligated into *BamHI*-digested λ 2004 (ref. 29). Recombinant phage were screened with a C_μ probe, C75pl.2 (indicated in **A**) (ref. 30). Positively hybridizing plaques were grown and restriction mapping carried out using the enzymes indicated. The presence of a V_H segment in the 5' *BglII* fragment was determined by hybridization with the V_H III probe, V_H 26-8 (ref. 13). R, *EcoRI*; H, *HindIII*; B, *BamHI*; Xb, *XbaI*; Bg, *BglII*. **C**, Southern filter hybridization of genomic DNA using C75pl.2; 10 μ g of placental DNA, Daudi (t8;14)³¹ or Raji DNA³² were completely digested with *BamHI*, fractionated on a 0.8% agarose gel and transferred to cellulose nitrate³³. The filter was hybridized with 10^6 c.p.m. ml⁻¹ nick-translated³⁴ C75pl.2 (specific activity $\sim 10^8$ c.p.m. μ g⁻¹) in 0.66 M NaCl, 0.2 M Na₂HPO₄, 0.006 M EDTA, pH 6.2, 10% dextran sulphate (Pharmacia), 1 μ g ml⁻¹ denatured *Escherichia coli* DNA, 1% sarkosyl, 0.2% bovine serum albumin, 0.2% Ficoll, 0.2% polyvinyl pyrrolidone³⁵. Hybridization was for 18 h followed by washing in 0.15 M NaCl, 0.045 M Na₂HPO₄, 0.001 M EDTA, 1% sarkosyl. Autoradiography was carried out for 18–36 h using preflashed film³⁶.



C_μ gene associated with V_H segment

B lymphocytes display allelic exclusion with respect to the immunoglobulin genes in that only one chromosome per cell generally undergoes productive rearrangement for immunoglobulin synthesis. As the C_μ gene is expressed in Raji cells and has also been implicated as the site of translocation in some BL cells^{11,12}, we investigated the C_μ gene in Raji DNA as a first step in the molecular analysis of the 8;14 translocation in these cells. Southern filter hybridization analysis using a C_μ probe (see Fig. 1A) revealed that Raji DNA contains only a single rearranged restriction fragment containing the C_μ gene (19 kilobases, kb) (Fig. 1C). This contrasts with the 16-kb fragment containing the germ-line C_μ gene in placental DNA and two rearranged fragments (17 and 12 kb) in Daudi cells (a different BL cell line also with t(8;14)) (Fig. 1C). It therefore seems likely that Raji cells contain only a single C_μ gene and that this is the active gene as these cells produce IgM. Positive proof of this was obtained by characterizing cloned DNA isolated from a phage library, prepared from Raji cell DNA, using the C_μ probe. Figure 1B shows the restriction map of these clones; the C_μ gene is located approximately 8 kb downstream of a segment hybridizing with the V_H III probe, V_H 26-8 (ref. 13). Evidently, therefore, this DNA region represents the active chromosome 14, from Raji cells, from which the μ heavy-chain mRNA is transcribed.

$c\text{-myc}$ and $C_{\gamma 1}$ genes are adjacent in Raji cells

Raji DNA has been shown to possess a rearranged $c\text{-myc}$ gene as well as the normal allele from chromosome 8 (ref. 11). Since Raji cells have only one C_μ gene this rearranged $c\text{-myc}$ cannot be found near the C_μ gene, as indicated for some other BL DNAs. Furthermore, restriction mapping data obtained with a variety of C_H gene probes indicated that Raji contained no obvious rearrangement of C_e or C_a but appeared to have a rearrangement of one of the C_γ genes (M.P.L., M. P. Lefranc, P.H.H. and T.H.R., unpublished). Therefore the possibility existed that the $c\text{-myc}$ rearrangement in Raji was associated with the putative rearranged C_γ .

To test this hypothesis, we isolated clones from the Raji phage library which were complementary to an avian $v\text{-myc}$ probe (see Fig. 2). Two types of clones were obtained. One type apparently contained the normal $c\text{-myc}$ allele from chromosome 8 (Fig. 2) as it overlapped extensively with the published version of the $c\text{-myc}$ restriction map^{14,15} and because it overlapped with an apparently non-rearranged $c\text{-myc}$ gene (also indicated in Fig. 2) isolated from a phage library prepared from JI DNA (a BL cell line containing a 2;8 translocation). Consistent with the published data, the Raji normal $c\text{-myc}$ gene contains two coding segments as defined by hybridization with the 5' and 3' $v\text{-myc}$ probes shown in Fig. 2.

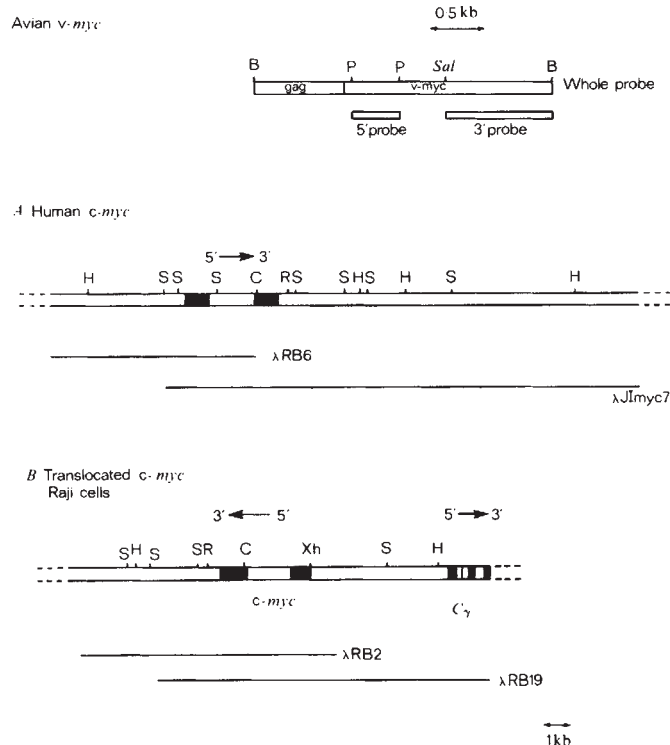


Fig. 2 Structure of $c\text{-myc}$ -containing recombinant phage clones from Raji DNA. The top panel shows the avian $v\text{-myc}$ probe plus the 5' and 3' probes used to orientate the coding region of the $c\text{-myc}$ gene. **A**, Structure of the normal $c\text{-myc}$ gene derived from clones obtained from the Raji library using Ryc 7-4 (clone λ RB6) (see Fig. 1) or from an analogous library prepared from a BL cell-line, JI (t2;8), probed with avian $v\text{-myc}$ clone (λ JImyc7). **B**, Structure of the translocated $c\text{-myc}$ gene from Raji cells. Two overlapping clones (λ RB2 and λ RB19) were mapped with the enzymes indicated and with respect to the hybridization of a human $C_{\gamma 3}$ probe and the 5'- $v\text{-myc}$ plus 3'- $v\text{-myc}$ probes. The 1-kb scale bar at the bottom of the figure refers to **A** and **B**; H, *HindIII*; S, *SacI*; C, *ClaI*; R, *EcoRI*; Xh, *XhoI*; B, *BamHI*; P, *PstI*. (Not all *ClaI* sites are indicated.)

from this cell line. In this BL example, the $c\text{-myc}$ and $C_{\gamma 1}$ genes are juxtaposed in the translocation involving the allelically excluded chromosome 14 while the C_μ gene has been mapped, in a different set of phage clones, in association with a V_H gene segment from the active chromosome 14. Northern hybridization analysis of the $c\text{-myc}$ mRNA shows that two size classes normally occur at high levels in BL and that these result from promoter sites upstream of a third previously unidentified exon in the human $c\text{-myc}$ gene.

Fig. 3 Alignment of the partial sequence of the γ subclasses. The top line shows the sequence of λ RB19 γ gene and below are indicated residues from the other γ subclasses which exhibit differences from the λ RB19 sequence. No differences compared with γ 1 were observed while multiple differences exist between the λ RB19 γ sequence and that of the other γ subclasses. The *Pst*I site underlined indicates the start of the hinge domain.

λ RB19	GCCCA	CCCCAAGGC	CAAACTCTCG	ACTCCCTCAG	CTCGGACACC	TTCTCTCTC	CCAGATTCCA	GTAAGTCGA	ATCTTCTCTC	<u>TGCAG</u>
γ 1										
γ 2		G		G			T	C	G	
γ 3				T	A		T		CTG	
γ 4					A		T		CTG	

The second type of clone containing *c-myc* sequences was the translocated *c-myc* gene. Restriction mapping and hybridization revealed the structure of this translocated *c-myc* gene. Figure 2 shows that in the translocation the other allele of *c-myc* has been joined to the C_H locus just upstream of a C_γ gene as determined by hybridization of a human C_γ probe, p3.6RH4.2 (ref. 16). The subclass of the C_γ gene involved was determined by DNA sequencing. We showed previously¹⁶ that all C_γ genes are present in small *Pst*I fragments of which one *Pst*I site occurs upstream of the C_H 1 domain and a second at the junction with the hinge segment. Thus these fragments carry an intervening sequence (IVS) between C_H 1 and the hinge; nucleotide sequences of this IVS adjacent to the hinge are characterized by subclass-specific residues thus allowing identification of each subclass. We therefore subcloned *Pst*I fragments from λ RB19 (*c-myc* + C_γ clone) in M13mp10 (ref. 17) and sequenced those clones¹⁸ which hybridized to the γ -gene probe, p3.6RH4.2. Figure 3 shows the sequence derived from such clones. The sequence indicated in the top line derives from the 3' end of the IVS between C_H 1 and the hinge in λ RB19 (the *Pst*I site underlined at the 3' end of these sequences represents the start of the hinge segment). The differences between this sequence and that of the γ subclasses is shown below the λ RB19 sequence. Although differences exist with γ 2, γ 3 and γ 4, there is exact correspondence to the γ 1 sequence, showing that the $C_{\gamma 1}$ subclass gene is involved in the Raji translocation.

The restriction map of the region containing *c-myc* and C_γ was examined using the 5' and 3' *v-myc* sequences to determine the orientation of *c-myc* relative to C_γ (the orientation of the latter within the clone is fixed by the presence of the right-hand *Hind*III site, which occurs on the 5' side of the C_γ genes¹⁶). This mapping showed that the *c-myc* and γ 1 genes are arranged in the opposite direction for transcription, with the *c-myc* upstream of C_γ (that is, 3'-5' *c-myc* 5'-3' $C_{\gamma 1}$) (Fig. 2). Hybridization experiments with the two *v-myc* probes detects two separate coding segments in the normal *myc* gene^{14,15} and both of these components are present in the *myc*/ C_γ translocated clones. Northern filter hybridization reveals the presence of at least another, third 5' exon which is present in the normal *c-myc* gene (see below).

The restriction map of the region adjacent to the 5' end of the translocated *c-myc* gene shows several unexpected differences from the normal *c-myc* gene. For example, the *Sac*I site at the 5' side of the *Cl*aI site is absent from the rearranged *c-myc* gene (Fig. 2) and nucleotide sequencing of the 5' end of the *c-myc* gene homologous to *v-myc* shows several point mutations (unpublished) compared with the normal *c-myc* sequence¹⁴. Further, as pseudogenes of *c-myc* exist¹⁵, we cannot formally exclude the possibility that the sequence variations occur because the translocated *c-myc* is a pseudogene. This sequence variation at the 5' end of the rearranged *c-myc* gene in Raji precludes a definitive localization of the translocation point in Raji cells. Nucleotide sequencing, however, shows that the methionine codon, identified by homology with *v-myc*¹⁴, is present in the translocated *c-myc* of Raji.

Does translocation of *c-myc* affect gene transcription?

If the specific translocations involving chromosome 8 (*c-myc*) in BL are required for generation of the leukaemia, the translo-

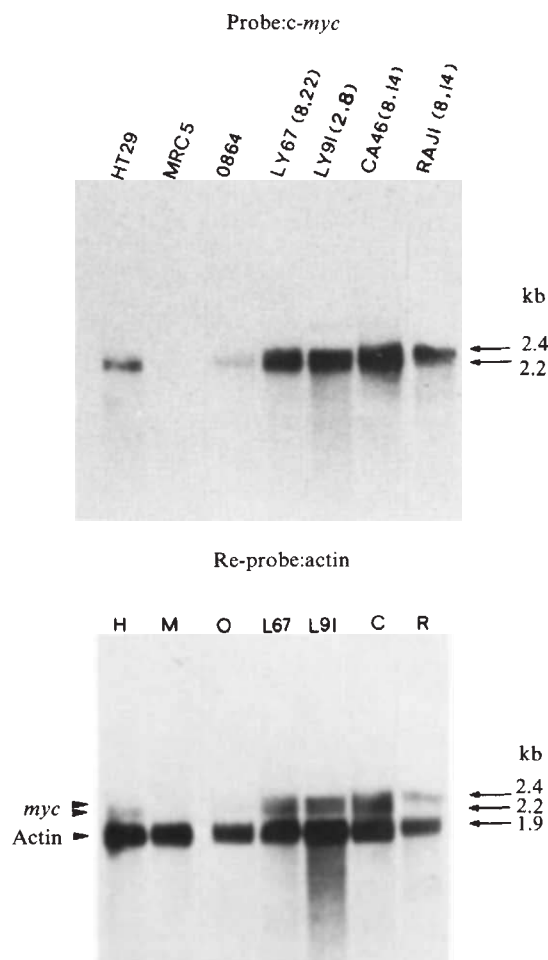


Fig. 4 Northern filter hybridization of mRNA from various cells with *c-myc* and actin probes. Top panel, autoradiograph of filters after hybridization to Ryc 7-4 (*c-myc*). Bottom panel, autoradiograph after re-hybridization to the actin clone. The rDNA hybridization profile (not shown) was used to estimate the contamination of the poly(A)⁺ RNA by rRNA and to calculate mRNA sizes assuming 28S = 5 kb, 18S = 1.8 kb. The cells used for RNA preparation (left to right) were HT29, MRC5, GM0864, LY67, LY91, CA46 and Raji.

Methods: RNA was extracted³⁷ from the cell lines indicated in the figure and poly(A) selection was carried out using oligo(dT) cellulose. Poly(A)-selected mRNA was quantitated by measuring optical density after affinity chromatography and equal amounts (5 μ g) of RNA were electrophoresed on a 1.4% agarose gel after glyoxylation³⁸. The RNA was then transferred to cellulose nitrate filters. Filters were probed sequentially with a nick-translated human *c-myc* clone (Ryc 7-4)²², a mouse actin clone and a *Xenopus* rDNA clone (given by Dr M. Davis); hybridization and washing were as described in Fig. 1 legend except that filters were washed in the equivalent of hybridization solution (minus probe) after hybridization with the actin or rDNA clones.

cation presumably in some way affects expression of the *c-myc* gene. We have studied *c-myc* gene transcription by Northern filter hybridization analysis, comparing the extent and size of *c-myc* transcripts in BL and other cell lines (Fig. 4). We compared the concentration of *c-myc* mRNA in four BL cell lines

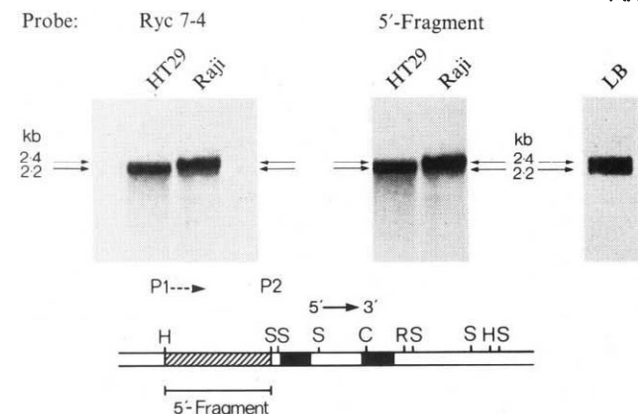


Fig. 5 Northern hybridization of poly(A)⁺ RNA from HT29 or Raji cells with third *c-myc* exon probe. Poly(A)⁺ RNA (5 µg) from HT29 and Raji cells (different RNA preparations from those used in Fig. 4) were used here to validate the difference in *c-myc* mRNA profiles) were fractionated and transferred to cellulose nitrate as described in Fig. 4 legend. The filter was cut into two pieces and hybridized separately to the human *c-myc* probe, Ryc 7-4 (ref. 22) (left-hand panel) or to the 5'-fragment probe (right-hand panel). The latter probe is the 3-kb *HindIII*-*SacI* fragment isolated from λ RB6 and indicated in the bottom part of this figure (5'-fragment) (also indicated are the putative transcription promoters P1 and P2). The extreme right-hand lane shows the hybridization of HMy2 (ref. 39) RNA (LB) with the 5' fragment cloned in an M13 vector (clone designated M13RB6HS1): this control RNA possesses approximately equal quantities of 2.4- and 2.2-kb *c-myc* RNA.

[Raji and CA46 both with t8;14, LY67 with t8;22 (ref. 2) and LY91 with t2;8 (ref. 2)], in a colon adenocarcinoma tumour line (HT29)¹⁹, a fibroblast line (MRC5) and a simian virus 40 (SV40)-transformed fibroblast line (GM864)²⁰. To control for possible artefacts in the observed levels of *c-myc* mRNA within particular RNA preparations, we routinely used a second probe to screen Northern filters—we used a mouse actin cDNA clone which should represent a fairly uniformly expressed mRNA species. The lower panel of Fig. 4 shows the results of reprobating a Northern filter with the actin probe (the actin-specific band is indicated). Approximately equal amounts of actin mRNA are present in each of the cell lines, indicating that the levels of *c-myc* RNA (shown in the top panel) are a true reflection of the actual mRNA level. An outstanding feature of the hybridization with *c-myc* (Fig. 4, top panel) is the high levels of *c-myc* mRNA in the four BL lines and in the non-BL line, HT29. The fibroblast line, MRC5, contains very little detectable *c-myc* mRNA while the SV40-transformed line shows a comparatively elevated level. As regards absolute levels of *c-myc* mRNA, although it is clear that all the BL lines examined have high levels of this mRNA, it is also apparent that non-BL lines (for example, HT29) have elevated *c-myc* RNA levels relative to, for example, fibroblasts. Indeed, two lymphoblastoid lines tested had levels of *c-myc* mRNA at least equivalent to BL levels (unpublished results). Evidently, therefore, different levels of *c-myc* expression are achieved by a variety of mechanisms in different cell lines types.

The mouse myeloma lines which show *c-myc* translocations appear to possess a new *c-myc* mRNA species^{21,22}. The data in Fig. 4 would seem to argue against this possibility in BL. However, we can clearly detect two sizes of *c-myc* mRNA (2.4 and 2.2 kb) in human cells; this is particularly apparent when Raji and HT29 RNA are compared with the actin band (Fig. 4, lower panel). In Raji mRNA most of *c-myc* consists of the 2.4-kb species while in HT29 the 2.2-kb species predominates (see also Fig. 5). This disparity in the relative amounts of the two transcript sizes is not the specific result of the *c-myc* translocation as, for example, CA46 which also contains a detectable rearrangement of *c-myc*¹², has approximately equal amounts of the two RNA species (Fig. 4). This is also true for LY67 and LY91 (Fig. 4) as well as Daudi RNA (unpublished).

There could be several origins for the two mRNA species. As there seems to be only one active *c-myc* allele^{14,15} plus several pseudogenes, the two mRNAs could arise on the active allele from different promoters, show different RNA splicing patterns, different transcription termination or even have different amounts of poly(A) tail. To investigate the first possibility, we examined the hybridization of a restriction fragment located upstream of the 5' exon homologous to *v-myc*. The restriction fragment selected (designated 5' fragment) was a 3-kb *HindIII*-*SacI* fragment (indicated at the bottom of Fig. 5) from λ RB6 (a phage clone containing the normal *c-myc* gene). The left-hand panel of Fig. 5 shows the differential expression of 2.4- and 2.2-kb *c-myc* mRNA in Raji and HT29 when probed with a *c-myc* probe (Ryc 7-4)²². When an identical filter was probed with the 5' fragment of normal *c-myc* (not included in the Ryc 7-4 clone) this fragment also hybridized to both the 2.4- and 2.2-kb mRNA species from Raji and HT29 cells (Fig. 5, right-hand panel). The extreme right-hand lane of Fig. 5 shows the hybridization of a cloned version of the 5' fragment to poly(A)⁺ RNA isolated from a lymphoblastoid cell line (LB): this RNA serves as a control in which approximately equal amounts of the two *c-myc* mRNA species are present. These observations lead to several conclusions:

(1) The normal, unrearranged *c-myc* gene on human chromosome 8 possesses at least one further exon which is located upstream (5') of the two exons that are homologous to chicken *v-myc*.

(2) This exon exists upstream of the methionine codon which is thought to represent the initiation codon of the *c-myc* protein (based on homology with the viral protein sequence¹⁴). This result implies that a different promoter site (designated P1 in Fig. 5) exists upstream of the site (designated P2 in Fig. 5) which was suggested to be a promoter based on its location near the 5' *v-myc*-homologous exon.

(3) The 2.4- and 2.2-kb species of *c-myc* mRNA both contain the 5'-fragment-homologous exon: the most likely explanation for these mRNA species is therefore differential RNA splicing of a common nuclear RNA precursor in much the same way as two forms of immunoglobulin heavy-chain mRNAs are derived by RNA splicing alternatives²³. In respect of synthesis of these two RNA species in BL cell lines, Raji seems to be exceptional: in all other BL cells which we tested, approximately equal amounts of each RNA were present. For example, CA46 (Fig. 5) and Daudi (unpublished data) have high levels of both mRNA types: interestingly, these cell lines differ from Raji in the precise nature of the 8;14 translocations. In CA46 the *c-myc* gene appears to be located near to *C_μ* (ref. 15) while in Daudi it is located at a large, unknown distance from *C_μ* (ref. 24); in neither case does the breakpoint appear to affect the 'promoter' sequence P2 (see Fig. 5) or P1 in the case of Daudi (although the effect of breakage in the P1 promoter in CA46 is unclear so far). Raji, therefore, may well be an abnormal case of BL in which the *c-myc* gene originally translocated to *C_μ* and then a secondary, pseudo-switch moved this *c-myc* from *C_μ* to *C_{γ1}*. If the translocation of *c-myc* is actually involved in carcinogenesis, it is likely that the former event would be the one concerned with cell transformation.

Translocation mechanisms involving *c-myc*

The results presented here show that in a BL cell line the *c-myc* oncogene has become associated with the *C_{γ1}* gene from chromosome 14. This association probably involves the 14q⁺ chromosome since the active chromosome 14 has a functionally rearranged *C_μ* gene and because in other cases of BL the *c-myc* gene has been shown to be associated with the inactive 14q⁺ (refs 24, 25). The results formally confirm the association of *c-myc* with *C_H* genes in BL and parallel the analogous studies of translocations in mouse plasmacytomas^{11,22,26-28}.

It seems likely that the nonrandom nature of BL translocations results from random events which occasionally involve the *c-myc* locus. When the latter is involved, neoplasia of the B-cell is initiated. Furthermore, it is probable, as was previously

suggested^{1,5,7}, that the rearrangement events associated with the immunoglobulin loci (particularly the *Igh* locus in which ~90% of BL translocations are seen) are the mediators of these rearrangements between chromosomes.

The absence of discernible new *c-myc* mRNA species in BL is a paradox and indicates a significant difference from the situation in mouse myelomas. A more subtle change may be an alteration in the RNA splicing patterns producing a change in ratio of the two mRNA species: such as effect might alter the concentration of *c-myc* protein in certain critical cellular compartments. This feature may be significant in leukaemogenesis. The translocated *c-myc* gene in the Raji clones is arranged in the opposite orientation for transcription to the *C_γ* gene in an analogous way to the translocated *c-myc* in mouse myelomas^{22,27}. Elevated expression from the translo-

cated *c-myc* gene might then result from specific enhancer sequences occurring upstream of *C_H* genes and which can operate in any orientation for genes in their locality. Studies of this mechanism will help to resolve some of the paradoxes indicated here and should yield information on the mechanisms for control of synthesis of antibody genes.

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Note added in proof: Recently Watt *et al.* (*Nature* **303**, 725–728 (1983)) have also presented evidence for a third exon in the human *c-myc* gene.

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LETTERS TO NATURE

Non-absorption dips in spectra of γ -ray bursts

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The spectra of more than 20 γ -ray bursts show dips in the 30–70 keV range^{1–3}, that have been interpreted as cyclotron absorption lines^{1,2}. The presence of these absorption lines, is one of the arguments in support of the belief that γ -ray bursts originate from neutron stars with very strong (10^{12} – 10^{13} G) magnetic fields¹. This interpretation, however, poses serious problems^{4–6}; for example, if the continuum is produced by optically-thin thermal cyclotron radiation it implies a magnetic field increasing with the distance from the surface of a neutron star⁵. Here we propose a different interpretation of the dips in the spectra of γ -ray bursts. We show that these dips could result from a superposition of two spectra: an optically thin thermal synchrotron spectrum with an (observed) turnover at about 55–110 keV and a softer one that is best fitted with a black body. We find black-body temperatures in the range of 7–12 keV. Due to poor statistics and the uncertain quality of the data⁷ our results should be considered only as tentative, our main objective is to point out that using a two-component interpretation for the dips in γ -ray bursts spectra could confirm the synchrotron mechanism.

Following Liang⁵ we fit the hard component of the spectrum with a function describing an optically-thin synchrotron emission of mildly relativistic thermal electrons⁸, but interpret the turnover preceding the dip ('absorption feature') as the

turnover due to synchrotron selfabsorption. The soft part (energies lower than the turnover energy E_T) is then treated as a superposition of an optically thick (approximately Rayleigh–Jeans⁹) selfabsorbed synchrotron spectrum with a spectrum that has to be determined by a fitting procedure (see Fig. 1).

For the soft part we have used as trial spectra: a black-body, a thermal bremsstrahlung $n_\gamma \sim E^{-1} \exp(-E/kT_e)$, and a synchrotron spectrum (the same form as for the hard part):

$$n_\gamma \sim \exp(-4.5E/E_c)^{1/3} \quad (1)$$

$$E_c = E_L T^2, \quad E_L = \frac{heB}{mc^2} = 11.6 \text{ keV} \cdot B_{12}, \quad T = \frac{kT_e}{mc^2}$$

(m and T_e are respectively the electron mass and temperature), $B_{12} = B/10^{12}$, and the photon spectrum density n_γ is in photons $\text{cm}^{-2} \text{s}^{-1} \text{keV}^{-1}$.

The appearance of a turnover in the spectrum does not immediately give the values of T and E_L , as one has to know first to what harmonic number $m_* = E_*/E_L$ corresponds the self absorption turnover at energy^{5,10}: $E_T \sim E_* (1 - T)$ where E_* is the energy at which the optical depth to the synchrotron selfabsorption $\tau(E_*) = 1$. The data are not good enough to give a precise value of m_* and in our analysis we have assumed $m_* = 4$, which is the lowest value of m_* for which the condition of applicability of (1) $E \gg E_L/T$ is satisfied. We have checked that the parameters of the fits are insensitive to the precise value of m_* .

The results of the fittings are presented in Table 1. The spectra were taken from refs 1, 2. (GB790307 = γ burst of 7 March 1979.) Obviously the samples are small, the results of the procedure should be treated with care, and the uniqueness of the fit can be questioned (for GB781115 in particular). Clearly, however, our results favour the black-body interpretation, particularly as regards the physics of the problem. In fact, models of γ -ray bursts describe a black-body spectrum more easily than the hard component^{11,12}.

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Table 1 Fits of the data with observations

	Black body		Synchrotron		Thermal bremsstrahlung	
	$kT_{bb}(\text{keV})$	χ^2	$\nu_c(\text{keV})$	χ^2	$kT_{Tb}(\text{keV})$	χ^2
GB790307	7.1 ± 0.9	1.0	1.98 ± 0.01	2.6	34.2 ± 8.4	1.8
GB791101	8.8 ± 1.0	1.4	0.21 ± 0.01	4.5	39.6 ± 9.0	3.3
GB781115a	11.7 ± 1.6	0.3	2.9 ± 0.1	0.1	121 ± 14	0.1
GB790622	8.7 ± 0.7	0.2	1.8 ± 1.0	2.1	67.4 ± 45	2.2

Table 2 Parameters corresponding to the four analysed spectra

	$E_T(\text{keV})$	T	B_{12}	L_{syn}^+ / A^*	$d_{syn}(\text{kpc}) / A^{1/2*}$	$kT_{bb}(\text{keV})$	L_{bb}^* / A^*	$d_{bb}(\text{kpc}) / A^{1/2*}$
GB790307	65	0.56	3.2	6.5×10^{42}	27	7.1	2.6×10^{37}	0.9
GB791101	90	0.43	3.4	3.4×10^{42}	45	8.8	6.2×10^{37}	1.6
GB781115a	110	0.50	4.7	1.7×10^{42}	43	11.7	1.9×10^{38}	2.4
GB790622	85	0.53	3.9	1.1×10^{42}	54	8.7	5.9×10^{37}	1.8

* A in km^2 .† L in erg s^{-1} .

The synchrotron luminosity is given by¹³:

$$L_{\text{Synch}} = 3.55 \times 10^{36} A T E_*^2 \left(1 + x_* + \frac{x_*^2}{2!} + \frac{x_*^3}{3!} + \frac{x_*^4}{4!} + \frac{x_*^5}{5!} \right) \text{erg s}^{-1} \quad (2)$$

where $x_* = (4.5 E_*/E_c)^{1/3}$, A is in units of km^2 .

In all cases the optical depth to Thomson scattering

$$\tau_{\text{cs}} = 1.4 \times 10^{-5} E_* T K_2(T^{-1}) e^{x_*}$$

(where $K_2(1/T)$ is a Bessel function) was of the order of 10^{-2} a value consistent with the uncomptonized shapes of the spectra.

In Table 2 we present the parameters corresponding to the four analysed spectra. The parameters d^2/A as deduced from the synchrotron component is 300–900 times greater than the d^2/A derived from the black body and this implies different areas for the synchrotron and black-body sources.

Note first, that equation (2) is definitely an overestimate of the synchrotron luminosity because it assumes emission from an isotropic maxwellian plasma⁵. One could, therefore, expect

that the actual values of d_{syn}^2/A would be substantially lower than those given in Table 2.

It is also plausible that the area of the black-body emitting surface is larger than the surface area corresponding to the synchrotron-emitting volume. We can envisage the following: the mechanism responsible for the γ -ray burst forms a hot 'column' of magnetically confined plasma over the neutron star surface. This happens both in the thermonuclear^{11,14} and asteroid capture^{12,15} models. The black-body radiation is then emitted from the column photosphere (and possibly from a hotspot on the neutron star surface) while the synchrotron radiation is emitted from the thin parts of the column by electrons accelerated in magnetic field reconnection¹¹ or other instabilities⁵ (in fact Woosley and Wallace¹¹ propose this kind of a two-component spectrum). In this case the volume involved in the synchrotron emission could be much smaller than the total volume of the column. Both the lower synchrotron emissivity and emitting volume would imply that the distances to the sources analysed here are ~ 1 rather than ~ 50 kpc.

The time variations of both the fluxes and the spectra should be rather complicated and the fact that some sources do not show spectral variation^{1,2,16} is more surprising than the observed rapid flickering^{3,17} since the later would be normal when shock waves, deflagrations, magnetic field instabilities and so on are involved in energy production and transport. It would be premature to discuss the rapid time variations of the dips in the spectra because of the controversial status of the SMM data⁷ and no significant dips in 'Signe' data. The disappearance of dips after 4–8 s can be explained by the increase of the 'photospheric' temperature that raises the soft component and, together with the hardening of the hard component, reduces or cancels the dip.

The soft component will probably persist for some time after the end of the γ -ray event but its temperature would fall to < 2 keV (ref. 11). A similar black-body temperature would be consistent with the hardness ratios as given in ref. 13 for the Vela X-ray events, but in the initial spectra of this events (as in the Apollo burst¹⁸) the dips are absent.

The spectrum of the ref. 19 which shows a dip at ~ 55 keV and a high-energy tail is probably produced by synchrotron, inverse Compton and annihilation emission of e^+e^- pairs²⁰ and not by mildly relativistic electrons and is different from spectra discussed here.

The strongest argument in favour of the two-component nature of the spectra of the γ -ray bursts is the persistence of a soft component between the peaks of GB7903524⁴. The real test of the present and other models will be provided by observations in the 1–20 keV range.

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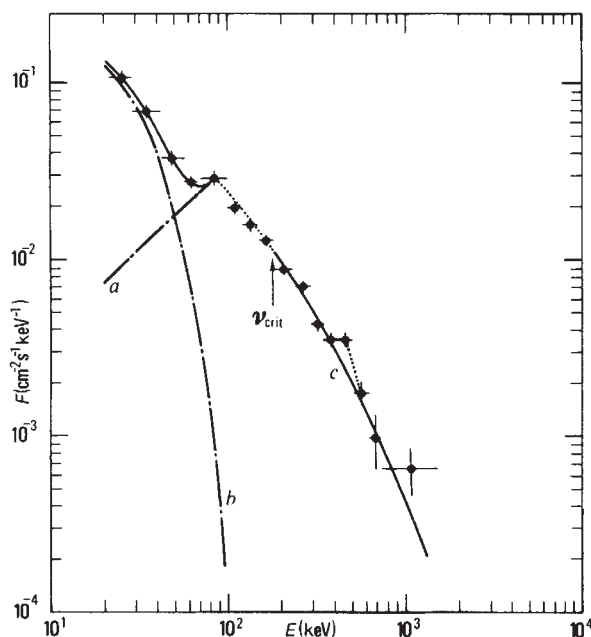


Fig. 1 The fit of the spectrum of GB 790622 (ref. 2). The curve a is a Rayleigh-Jeans spectrum, the curve b is a black-body spectrum, the curve c is a synchrotron spectrum^{5,8}. At $\nu_{\text{crit}} \approx \nu_*$ the emission begins to be optically thick. The turnover is at 85 keV.

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Satellite microwave radiances correlated with radar rain rates over land

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Satellite methods for the measurement of precipitation have used images obtained at visible, IR and microwave frequencies. Visible/IR methods infer precipitation from the appearance and behaviour of clouds. Microwave methods are more direct because the microwave radiation upwelling from the Earth is affected more by rain drops than by cloud droplets. These radiances viewed by a sensor outside the atmosphere are presented here as brightness temperature, the product of the thermodynamic temperature and emissivity of the surface viewed, modified by the intervening atmospheric constituents (water droplets, water vapour, gaseous absorption, and so on).

The radiative transfer calculations of Savage and Weinman¹ for a rain cloud over land show that the upwelling radiation at 37 GHz should be sensitive to changes in rain rate up to rates of 20 mm h⁻¹. When the Earth is viewed obliquely in vertical and horizontal polarizations, the radiation from standing water is highly polarized (wet soil and vegetation less so) whereas radiation from a rain cloud is only slightly polarized. Thus, as Weinman and Guetter² and Rodgers *et al.*³ demonstrated, it is possible to distinguish between rain, lakes, and dry ground at 37 GHz. However, Rodgers *et al.* also found that it is difficult to distinguish between rain and wet ground. Furthermore, dew-covered vegetation had the appearance of rain, and all distinctions between rain, wet land, dry land, and water disappeared for surface temperatures between 5 and 15 °C. Partly because of these results—which were obtained from a single frequency (37 GHz)—and partly because the emissivity of land at microwave frequencies ranges over a factor of two, the prospects of measuring rain rate over the continents by means of satellite-borne microwave radiometers generally had been discounted^{4,5}.

A recent instrument, the Scanning Multichannel Microwave Radiometer (SMMR)⁶ on the Nimbus 7 and Seasat satellites measures measures perpendicularly polarized antenna temperatures at five frequencies, that is, 37, 21, 18, 10.7 and 6.6 GHz. Its scan along a conical surface results in a constant incidence angle of 50° at the surface of the Earth. Horizontal resolution at the Earth varies from a roughly circular footprint of ~20 km diameter at 37 GHz (a slight improvement over previous instruments) to about 70 km at 10.7 GHz. Rodgers *et al.*

successfully distinguished between rain, wet ground, and dry ground in data from SMMR for several orbital passes over the United States when data at 37, 18 and 10.7 GHz were used, and when surface temperatures were >15 °C. Taking their work one step further, rain rates derived from weather radar have been compared with brightness temperatures measured by the Nimbus 7 SMMR. In data collected over the central United States during the warm season, microwave radiances have been found to explain a relatively large percentage of the variance of the radar rain rates, over a wider range of rates than previously thought to be impossible.

Like SMMR, radars provide an instantaneous view over large, spatially continuous areas. The WSR-57 radars in the US National Weather Service network are maintained according to uniform procedures⁸, including calibration checks made weekly or when there is a threat of severe weather. Radar plan position indicator (PPI) photographs are taken routinely and archived on microfilm. Relationships between the radar-observed reflectivities and rain rates have been adopted for these radars based on theory and extensive observation. The six reflectivity levels displayed by the WSR-57 were assigned average rain rates⁸ of 4, 17, 42, 85, 147 and 190 mm h⁻¹. The errors in rain rates inferred from radar measurements are caused by differing reflectivity–rain rate relationships among and even within storms, calibration changes, radar beam height change with distance from the transmitter, attenuation of the radar beam by heavy rain, evaporation of rain, rain travelling with the wind before reaching the ground, and false echoes due to anomalous propagation. These errors restrict the typical accuracy of radar rain rate measurements by research radars to a factor of 2 (ref. 9). Nevertheless, for comparison with SMMR data, the temporal and spatial sampling similarities between the SMMR and the WSR-57 outweigh any advantage in point accuracy afforded by available networks of rain gauges. Hence, the present study only considers rain rates inferred from WSR-57 radars.

PPI pictures were inspected on a microfilm reader. To be selected for digitizing, a picture had to show good time continuity of echo movement (thus excluding most cases of anomalous propagation), have an antenna elevation angle of <2°, and be properly exposed. Only data within 230 km of the radar site were included. Most of the digitized pictures were taken within 3 min of the SMMR observation times; all were within 6 min.

Selected pictures were registered on a 20 km grid. The fraction of each 20 × 20 km bin covered by each intensity level was visually estimated and the corresponding rain rates were weighted by areal coverage to get an average bin rain rate. Based on the operational nature of the WSR-57s, inter-radar differences, and the manual digitization of varying quality photographs of radar scopes that display only six discrete reflectivity levels, most of the resulting rain rates are within an error range of ±60%.

Table 1 Variables in the order chosen by the stepwise regression procedure

Index (i)	Frequency (GHz)	Polarization	Coefficient (a _i)	Multiple correlation coefficient	% Variance explained
0			32.7		
1	37	V	-0.363	0.579	34
2	21	V	0.172	0.710	50
3	21	H	0.200	0.724	52
4	37	H	-0.414	0.759	58
5	18	V	0.116	0.763	58
6	10	H	-0.214	0.772	60
7	18	H	0.385	0.790	62

Also shown are the coefficients of the regression equation. The units of a₀ are mm h⁻¹ and all other a_is have units of mm h⁻¹ K⁻¹. Only those data where the 37-GHz polarization was <15 °C and where the 37-GHz horizontal brightness temperature was <280 K are included, leaving a sample of 3,813 cases.

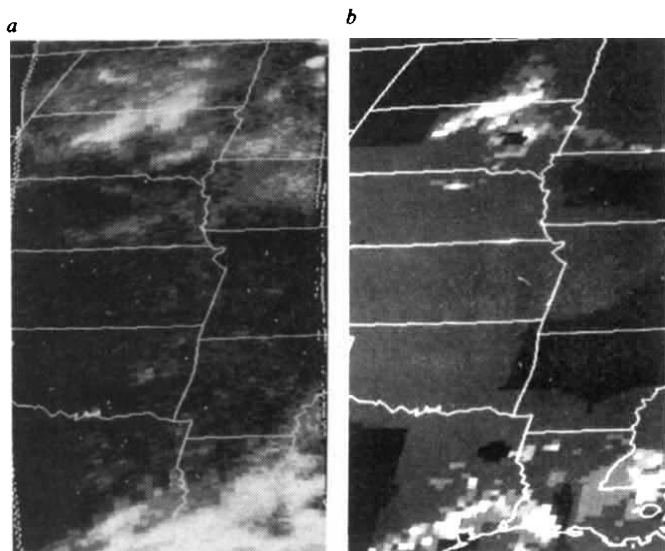


Fig. 1 SMMR 37-GHz vertically polarized image of the central United States at 1730 GMT 13 July 1979 (*a*), and the corresponding digitized radar image (*b*). The range of brightnesses in *a* represents temperatures from 229 to 290 K. The range of brightnesses in *b* represents rain rates from zero (light grey) to 45 mm h⁻¹ (white).

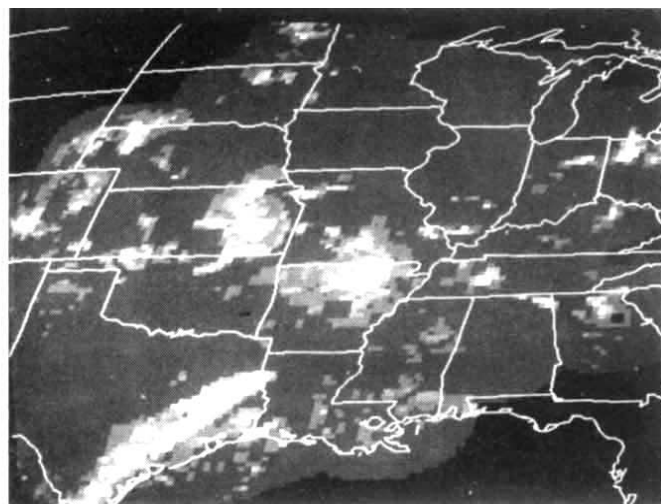


Fig. 2 A composited radar image comprised of all the rain systems used in the algorithm development. The range of brightnesses represent rain rates from zero (light grey) to 80 mm h⁻¹ (white).

The analysis included data from the region east of the Rocky Mountains and west of the Appalachian Mountains. We chose one satellite orbit from early May, and eight orbits from a three-week period during late June and early July of 1979. Four of the nine orbital passes occurred near local noon, and five near midnight. The orbit times chosen coincided with the most active rain systems during the period, ensuring that a significant number of rain storms would be available, as there was always an ample number of cases with no rain.

Microwave brightness temperatures and the digitized radar rain rates were entered into the University of Wisconsin's Man-computer Interactive Data Access System, McIDAS¹⁰. SMMR data displayed as an image on a TV screen were checked

for correct geographical placement, using the contrast at 37 GHz between water bodies and land as a guide. The largest repositioning of the data did not exceed one 37-GHz footprint (20 km). Figure 1*a* is an example of a 37-GHz vertically polarized image over the central United States. Several cold (bright) areas resemble rain areas in the corresponding radar image (Fig. 1*b*). Other cold spots correspond to lakes and the Gulf of Mexico. Figure 2 shows a composited radar image of all the rain systems included in the analysis. The brightest areas correspond to the heaviest rain rates (up to 80 mm h⁻¹).

Image sets consisting of the SMMR and digitized radar rainfall data were sampled on a 40×40 km grid. Only data within the digitized radar scans were included. A 40-km grid area

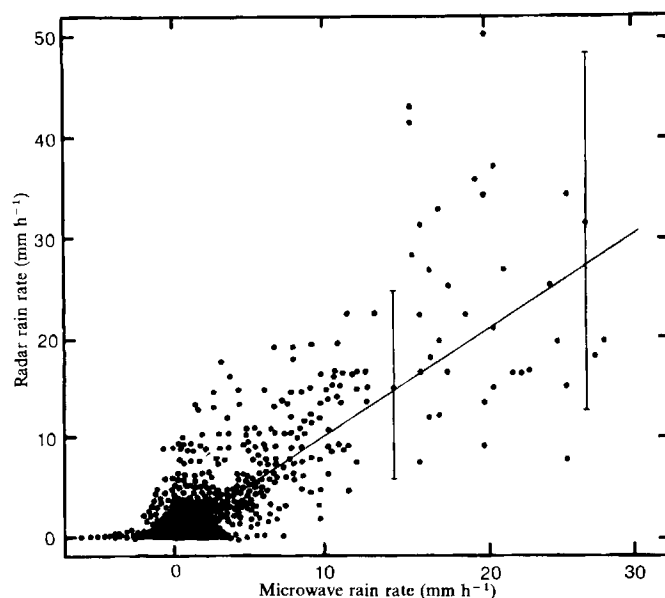


Fig. 3 A scatter plot of the radar-measured rain rates versus the microwave algorithm-derived rain rates for the dependent (training) data set. This set is comprised of 3,813 data points representing colocated radar and microwave data, each averaged over 1,600 km² areas. Sample error bars are shown for the rain rates, as well as a line of slope 1, which is also a least-squares fit.

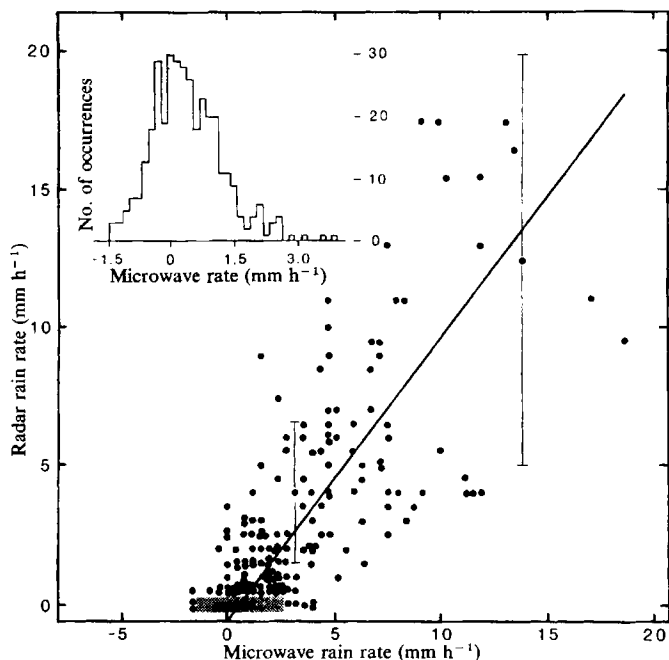


Fig. 4 A scatter plot of the radar versus microwave-measured rain rates for the independent algorithm test, with a line of slope 1. Sample error bars are shown for the radar rain rates. The wide shaded area at the origin contains ~100 points while the small shaded area contains 270. The inset shows a frequency histogram of the predicted rain rates for radar rain rates of zero, in intervals of 0.15 mm h⁻¹.

average was calculated from each image, resulting in a set of 4,663 'records', each consisting of averaged SMMR and radar data.

Rain never occurred in these warm season cases when the horizontally polarized 37-GHz brightness temperature was above 280 K. Also, cases of wet land or water bodies could be screened out by accepting only those records with a polarization difference between the vertical and horizontal 37-GHz averages that was $<15^\circ\text{C}$. Lastly, microwave data at 37 GHz were found to be approximately linearly related to rain rate, with the lower brightness temperatures corresponding to the heavier rain rates. This linear relationship held for rain rates up to at least 40 mm h^{-1} , due to unusually low brightness temperatures associated with the heavier rain rates. Spencer *et al.*¹¹ found that the cold 37-GHz temperatures (as low as 163 K) are probably due to backscattering of upwelling radiation by a layer of large ice particles. This layer is created and supported by strong thunderstorm updrafts, a condition required for the production of heavy rain. A similar result has been found in aircraft data obtained at 92 GHz (refs 12, 13).

A stepwise multiple-linear regression procedure¹⁴ was used to explore the extent to which the various channels were capable of supplying independent rain rate information. The rain rate, R , derived from microwave brightness temperatures, T_i , can be given by

$$R = a_0 + \sum_{i=1}^7 a_i T_i \quad (1)$$

The parameters derived from this regression analysis are shown in Table 1. Those terms with negative coefficients (37V, 37H, 10H) provide information on upwelling radiation from land that is attenuated by rain. Terms with positive coefficients (21V, 21H, 18V, 18H) represent a measure of the land background temperature in the vicinity of the rain cell. The brightness temperatures represented by these terms respond less to radiation from rain because the radiometers have coarser spatial resolution and measure radiation at longer wavelengths compared with the drop size of rain. Both rain-attenuated and land background brightness temperature measurements are needed because the estimate of rainfall is based on the extent to which the radiation from land is attenuated by the rain. A display of the radar-measured and algorithm-estimated rain rates for the training data set (Fig. 3) illustrates the ranges of rain rate that were involved in the algorithm development. Note that of the 3,813 data points, three radar-observed cases of rain rate between 40 and 50 mm h^{-1} were not well explained by the microwave data. However, Spencer *et al.*¹¹ found that 37-GHz observations on a smaller spatial scale (400 km^2) were generally able to distinguish rain rates well above 40 mm h^{-1} in conjunction with heavy thunderstorms. The multiple correlation coefficient of this algorithm is 0.79. Considering the errors inherent in the manual digitization of radar PPI scope photographs, this is a remarkable result. It was found that an insignificant increase in correlation resulted from inclusion of nonlinear terms in the regression procedure.

We applied this algorithm to an independent set of SMMR data observed at the time represented in Fig. 1. SMMR-derived rain rates were compared with the corresponding radar rain rates shown in Fig. 1b. The relationship between the radar-observed and microwave-predicted rain rates is shown in Fig. 4. Note that the algorithm slightly underestimates the rain intensity at higher rain rates for this test data set. Figure 4 also shows a frequency histogram of the algorithm-predicted rain rates for radar rain rates of zero. Most overestimates are $<1.5\text{ mm h}^{-1}$. The underestimates would be set to zero in any operational use of the algorithm; however, they illustrate the range of uncertainty that can be expected in low rain rate cases. A correlation coefficient of 0.80 for this independent test illustrates the algorithm's usefulness in estimating quantitative rain rates over land during the warm season.

Whether the relationship found here applies to other regions or times of the year remains to be investigated. However valid

equation (1) might be, the sampling frequency of the present observing system on Nimbus 7 (twice per day, every other day) is insufficient for the routine measurement of daily rainfall. To achieve that goal more frequent microwave observations, either from additional polar orbiters or a geostationary satellite, would be required.

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Defects in nsutite ($\gamma\text{-MnO}_2$) and dry-cell battery efficiency

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Nsutite is a major manganese oxide that occurs in mineable quantities in several terrestrial localities¹ and has also been reported to occur in manganese nodules². It has industrial importance^{3,4} since the intergrowth mineral and its synthetic analogue ($\gamma\text{-MnO}_2$) are used as the cathodic material in dry-cell batteries. The effectiveness of the material in batteries varies widely, and the physical cause of this variation is poorly understood. Structure imaging by high-resolution transmission electron microscopy (HRTEM) reported here reveals a variety of new structures and defects that may control battery efficiency by allowing increased diffusion rates.

Nsutite is one of three related families of tunnel structures that occur in the manganese oxides. In previous work we have determined the structural features of the other two families—hollandite-romanèchite⁵ and todorokite⁶. Structure imaging by HRTEM showed hollandite-romanèchite to consist of a coherent intergrowth of similarly-sized tunnel or framework structures that have a double chain of manganese-oxygen octahedra in common. Analogously, todorokite consists of an intergrowth of tunnel structures having a common triple chain of manganese-oxygen octahedra. Previously, nsutite had been shown by X-ray diffraction (XRD) measurements to consist of elements of two structures that intergrow by sharing a common single chain width⁷. The two key nsutite structure types are sketched in Fig. 1. Pyrolusite ($\beta\text{-MnO}_2$) consists of single chains of octahedra arranged as in Fig. 1a, whereas ramsdellite (MnO_2) has a structure of linked double chains arranged as in Fig. 1b.

Many XRD patterns of nsutite closely resemble those of ramsdellite^{7,8}. The nsutite structure was envisioned to consist mainly of double chains with randomly interspersed single chains of pyrolusite⁷. No superstructures were found in XRD

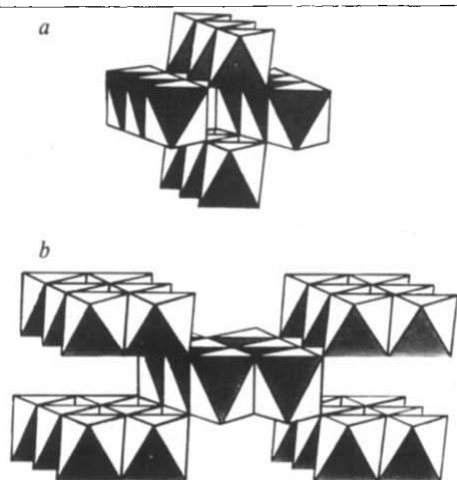


Fig. 1 Simplified diagram of structures of: *a*, Pyrolusite; *b*, ramsdellite. The basic unit is a manganese-oxygen octahedron. The octahedral chains are actually at inclined angles to one another, as shown in subsequent projections. Intergrowth of the two structures forms nsutite.

patterns of nsutite, and so regular alternation of single and double chains was not predicted.

Details of the structure of nsutite are of particular interest in the analysis of battery performance. Naturally-occurring nsutite and its electrolytically and chemically formed analogues are used as the cathodic material in many cylindrical and flat batteries⁹ and are being considered for use in button batteries¹⁰. An important feature of good battery material is its ability to allow proton diffusion to the interiors of individual manganese oxide grains¹¹. Materials having pure single- or pure double-chain structures are not as effective in this process as the intergrowth of the two found in nsutite¹². This difference in efficiency may have some relation to the fact that pyrolusite and ramsdellite are anhydrous whereas nsutite commonly has a significant water content¹¹. It has also been speculated that structural defects in nsutite facilitate enhanced proton diffusion and hence greater efficiency^{11,13}. We now provide evidence for such defects.

We examined nsutite from its type locality of Nsuta, Ghana, from Molango Piedras Negras, Hidalgo State, Mexico and from Honshu, Japan. Samples were supplied by J. White at the United States National Museum where the catalogue numbers are USNM R16115 (Ghana), USNM 119336 (Mexico) and

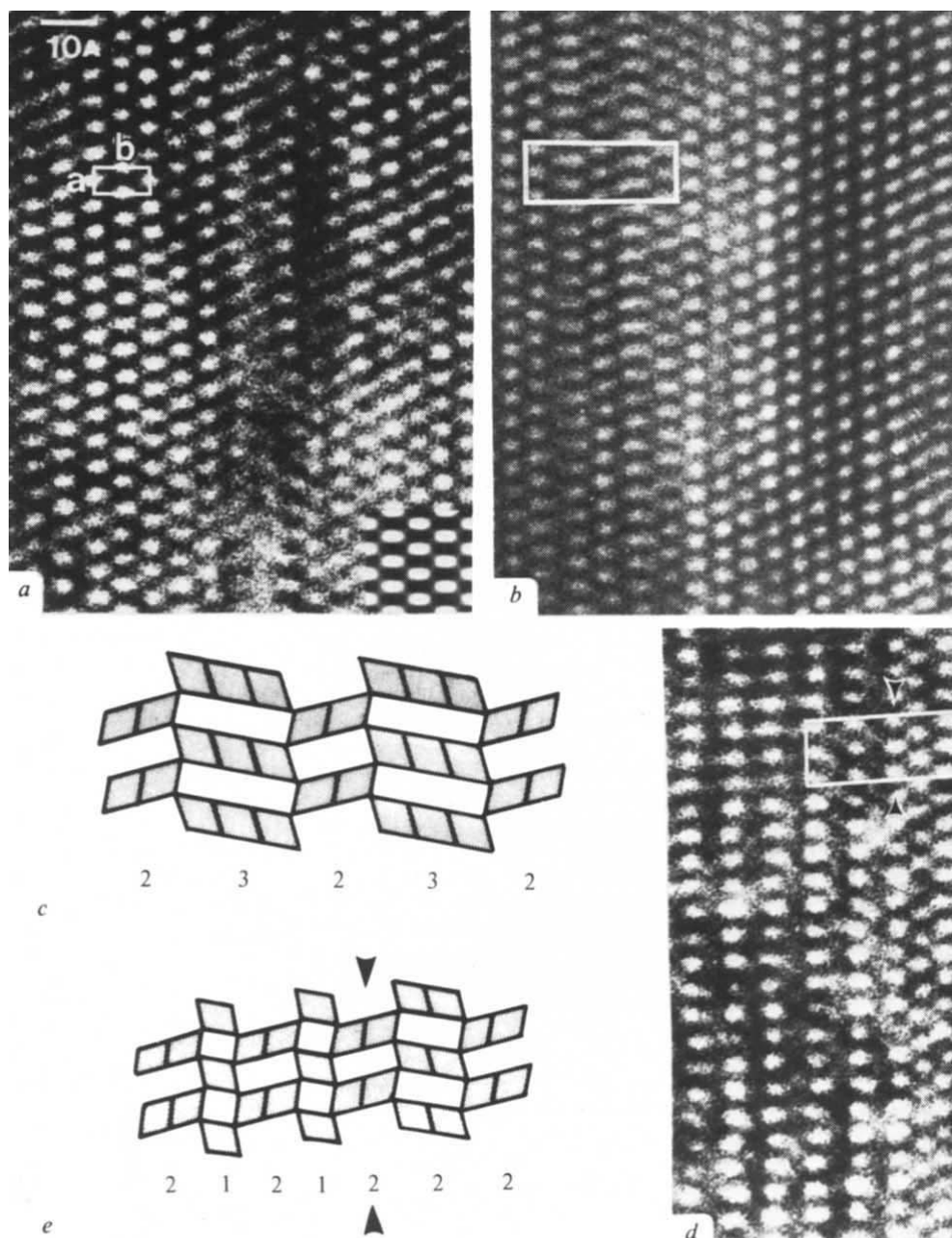


Fig. 2 HRTEM images and sketches of coherent structures within nsutite. All structures are viewed down the tunnel direction and have the same scale as indicated in *a*. *a*, Image of a double-chain (ramsdellite) region in nsutite (from Piedras Negras, Mexico). The inset in the lower right-hand corner is a computed image. Dark areas correspond to projected double chains of manganese-oxygen octahedra, the white 'spots' correspond to open tunnel regions. A unit cell of ramsdellite structure is outlined in white. *b*, Image of double-chain region with included triple chains (sample from Nsuta, Ghana). The outlined region is sketched in *c*. Each diamond-shaped box in *c* is a representation of a projected single chain of octahedra. The numbers in *c* indicate the number of edge-linked octahedral chains. *d*, Image of coherent intergrowth of two structural types (sample from Piedras Negras, Mexico). To the left of the arrow is a structure consisting of alternating single and double chains. Narrow slabs of this structure are intergrown with regions of double-chain material. A segment of this image is sketched in *e*. Note that at this resolution the tunnels in slabs of single chains are not resolved, but their presence can readily be inferred by the separations and position of the double chains.

USNM 118809 (Japan). We were successful in imaging down the tunnel direction for the Ghana and Mexico samples. Experimental procedures are as described elsewhere^{5,6}.

The bulk of material imaged in the Ghana and Mexico samples consists mainly of the double-chain structure as shown in Fig. 2a. In this and in subsequent images, the structure is oriented so that we are viewing down the tunnel direction. At an instrumental defocus of $-900 \text{ \AA}$, the dark regions correspond to projected manganese-oxygen octahedra. A computed image is included in the lower right-hand corner of Fig. 2a supporting this interpretation (the image was computed by methods outlined by O'Keefe *et al.*¹⁴ for an instrumental voltage of 100 kV, a spherical aberration constant of 2.2 mm, a depth of focus of $100 \text{ \AA}$, a specimen thickness of 20 unit cells ($57.32 \text{ \AA}$) and a defocus of $-900 \text{ \AA}$). In one sample grain, we found triple octahedral chains intergrown with the double chains, as shown in Fig. 2b. The structure within the white box is sketched in Fig. 2c. The presence of these larger tunnels in nsutite presumably affects the efficiency of proton diffusion.

A prominent structural feature in regions of some grains is the regular alternation of single and double chains, as shown to the left of the arrows in Fig. 2d. Although the 1×1 tunnel of pyrolusite is not resolved in this image, it can be deduced

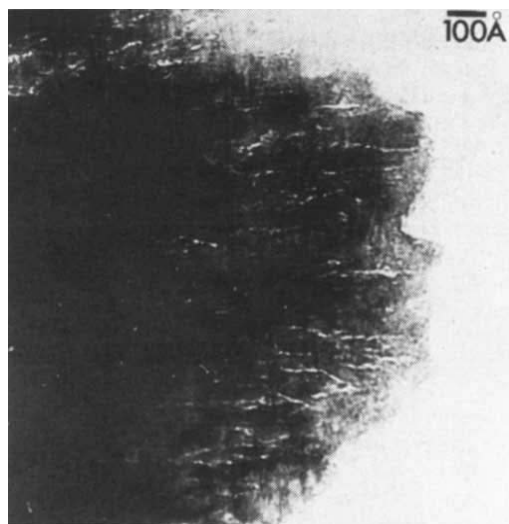
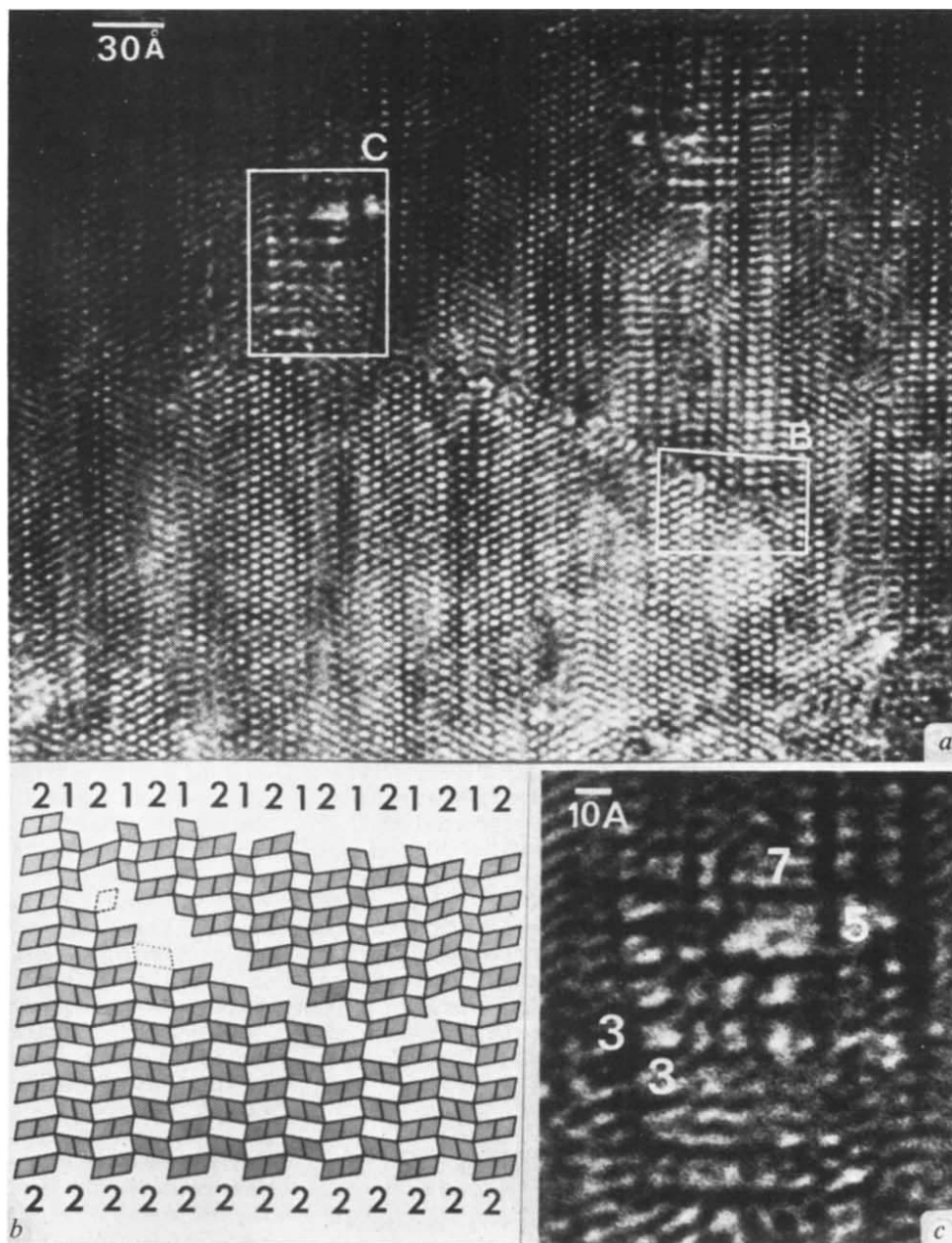


Fig. 3 Low-magnification image of defects in nsutite (from Nsuta Ghana). The image is taken in strongly defocused conditions to emphasize the defects.

Fig. 4 Images and sketch of a disordered region of nsutite (from Piedras Negras, Mexico). *a*, Overall view of the region. Several defects are present. Particularly striking is the diagonal fault in the centre of the image. *b*, An interpretation of the boxed region labelled B in the image. The upper portion consists of alternating single and double chains. The lower portion is of pure double-chain material. The exact nature of the structure around the defect is uncertain. The dashed single chain is possibly present. The dotted double chain is possibly absent. *c*, Enlargement at a different defocus of the TEM of the region labelled C in *a*. The material corresponds to todorokite. A tunnel structure with three octahedral chains on each side is labelled on the lower left. A large irregular tunnel is present on the upper right and has two sides labelled 5 and 7.



by the spacing of the 1×2 tunnels. Such ordered regions are not extensive ($< 100 \text{ \AA}$ in width) but are common in the grains we have thus far imaged. Regular alternation of different chain widths is unusual in the other manganese oxide intergrowth families. In the hollandite-roman  chite and todorokite families, intergrowths are random with no short-range ordering of tunnel widths. The alternating structure in nsutite can intergrow coherently with double-chain material by sharing a common double-chain width (Fig. 2d, e).

We have shown new structures and defects that are coherent with the bulk structure. However, many regions also contain disruptions in the structure. These disruptions can easily be seen in low-magnification, defocused images of nsutite. Figure 3 is an image of a region of nsutite taken in highly defocused conditions. The white lines are the result of Fresnel diffraction from the defects. A high defect density is evident in this image.

An interpretation of one type of noncoherent defect can be derived from the image of Fig. 4a, in which a diagonally trending defect is evident. Concentrating on the region within the box labelled B, we see that the upper region consists of alternating single and double chains and the lower region contains pure double-chain material. It is not obvious how the structures join. However, they clearly do not meet coherently, and there may be a significant gap in the 'seam'. A possible interpretation of the lower, right-hand end of the defect is sketched in Fig. 4b. Clearly, the ideal structures with this defect coincide only at every twelfth double chain of the pure double-chain material, indicating a type of semicoherent intergrowth. Such semicoherent interfaces may provide 'super tunnels' for proton diffusion and water transport, and so it is possible that such defects are an important feature of good battery material.

Another interesting feature is observed in Fig. 4a. When the region labelled C is imaged at a different defocus of the TEM, Fig. 4c, this feature is shown to be an inclusion of todorokite in nsutite. At the lower left-hand side of Fig. 4c is a todorokite structure with three octahedral chains on each side. At the upper right-hand side is a huge, irregular tunnel with one side consisting of five octahedral chains and another of seven octahedral chains. This is the largest manganese oxide tunnel structure known to exist. The todorokite structures are presumably filled with water molecules and large cations. The presence of water in todorokite regions may explain chemical analyses of nsutite showing significant water content^{8,15}. Such larger tunnel structures in nsutite were predicted by Burns and Burns¹³. It is interesting that the todorokite in Fig. 4 occurs near the nsutite defect, and it may be that todorokite is an alteration product of nsutite. We would expect alteration to occur along defects that facilitate water and cation diffusion. In some cases, however, inclusion of todorokite occurs near no apparent other defects in the nsutite.

Several of the new structural features found in natural nsutite—the triple chains, the semicoherent intergrowths, and the presence of todorokite—may have bearing on the activity of proton diffusion in battery material. Structure imaging of battery manganese oxides, both before and after their discharge, may provide further insight into the discharge process. HRTEM is a useful supplement to XRD methods for study of this complex intergrowth material.

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Anomalously old ⁴⁰Ar–³⁹Ar ages of Antarctic meteorites due to weathering

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In 1969, the Japanese Antarctic Research Expedition found nine meteorites on bare ice near the Yamato mountains, East Antarctica¹. Since then, >5,000 meteorites have been found in Antarctica by meteorite search teams^{2,3}. They are generally estimated to have been exposed on the ice sheet surface for $>10^3$ – 10^4 yr (ref. 4), and have been shown to have been subject to weathering effects^{5–8}. By comparing the ⁴⁰Ar–³⁹Ar age patterns for the inner and outer portions of two Antarctic meteorites, we have found that the oxidized outer portions show anomalously old ⁴⁰Ar–³⁹Ar ages which exceed 4,600 Myr in the intermediate temperature fractions of around 800–1,000 °C. This is contrary to the generally expected tendency as a result of Ar loss caused by weathering of a sample. Because old ⁴⁰Ar–³⁹Ar ages are accompanied by an increase of ³⁶Ar, we attribute the anomalous ages to the incorporation of terrestrial atmospheric Ar in some phase like haematite which might be changed from goethite during neutron irradiation.

To examine the effect of weathering on the ⁴⁰Ar–³⁹Ar ages of Antarctic meteorites, two relatively large meteorites, collected at Allan Hills, were selected. The sample ALH-761 of L6 type is a block with a diameter of ~20 cm. From it, the outermost portion, 61, the intermediate portion, 62, and the innermost portion, 64, were prepared (Fig. 1). The samples ALH-761, 60 and 67 were chemically analysed and show no distinct difference in their chemical compositions except for H₂O(+) contents⁶. The apparent difference among them is that the outer portion contains more rusty oxidized materials which are amorphous and regarded to be goethite under microscopic observations. The H₂O(+) contents in each portion reflect the above tendency as shown in Fig. 1. All samples were vacuum sealed in quartz vials together with age standard samples (MMhb-I)⁹ and irradiated for 25 days by JMTR (Japan Material Test Reactor) with the total fast neutron flux of 10^{19} neutrons cm⁻². The experimental procedures and all necessary correction procedures are almost the same as reported previously^{10–12}. Nine temperature steps (600–1,300 °C with 100 °C intervals and then steady at 1,550 °C for 45 min) were applied. The results are summarized in Fig. 2 and show approximately similar ⁴⁰Ar–³⁹Ar age release patterns except for the 1,000 °C fractions. The apparent decrease of ⁴⁰Ar–³⁹Ar ages at higher temperatures would be attributed to recoil effects¹³. The large variation for the highest temperature fractions might be attributed to the insufficient blank and/or Ca corrections. The outer portion ALH-761, 61 shows slight increase in the ⁴⁰Ar–³⁹Ar ages from the lower to higher temperature fractions.

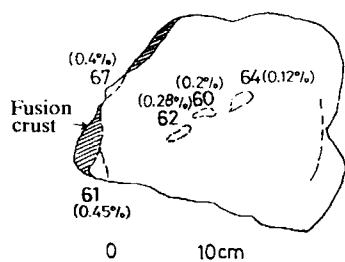


Fig. 1 The sample ALH-761 (L6) where numbers indicate each portion. The portions 60 and 67 were used for chemical analyses (H. Haramura, personal communication) and the portions 61, 62 and 64 for ^{40}Ar - ^{39}Ar analyses. The values in parentheses indicate $\text{H}_2\text{O}(+)$ contents for each portion.

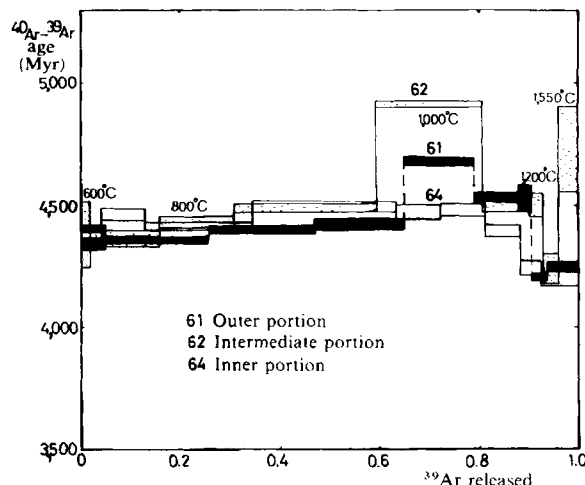


Fig. 2 ^{40}Ar - ^{39}Ar age diagrams for the sample ALH-761. The uncertainties indicate 1σ .

The innermost portion ALH-761, 64 shows a plateau age of $4,487 \pm 50$ Myr for 900–1,100 °C fractions, which covers ~46% of the total released ^{39}Ar . The outer and intermediate portions ALH-761, 61 and 62 show the ^{40}Ar - ^{39}Ar ages of $4,679 \pm 18$ Myr and $4,912 \pm 17$ Myr, respectively, in the 1,000 °C fractions. Since these old ages only appear in the oxidized portions, they cannot be regarded as reflecting the incorporation of some exotic materials that might have older origin than the Solar System. If we compare the release patterns of Ar components, the old ^{40}Ar - ^{39}Ar age is accompanied by the anomalous increase of ^{36}Ar as shown in Fig. 3b. No such increase of ^{36}Ar is observed in the intermediate temperature fraction for the sample ALH-761, 64 (Fig. 3a). Although no plot is shown here, a similar increase of ^{36}Ar as for ALH-761, 62 is observed for the sample ALH-761, 61. Slight deviation in the released fraction for ^{40}Ar is also observed at 1,000 °C for the sample ALH-761, 62. If we assume that all the observed ^{36}Ar in this fraction for ALH-761, 61 and 62 reflect the terrestrial atmospheric Ar and recalculate the ^{40}Ar - ^{39}Ar ages, they decrease to the values of about 4,400 Myr. This suggests that most ^{36}Ar in this fraction is probably of terrestrial-atmospheric Ar, including a small fraction of ^{36}Ar of trapped and/or cosmogenic origin. Hence, old ^{40}Ar - ^{39}Ar ages observed in the 1,000 °C fraction for the oxidized portions of the sample ALH-761, 61 and 62 have probably been caused by the addition of atmospheric ^{40}Ar to this fraction.

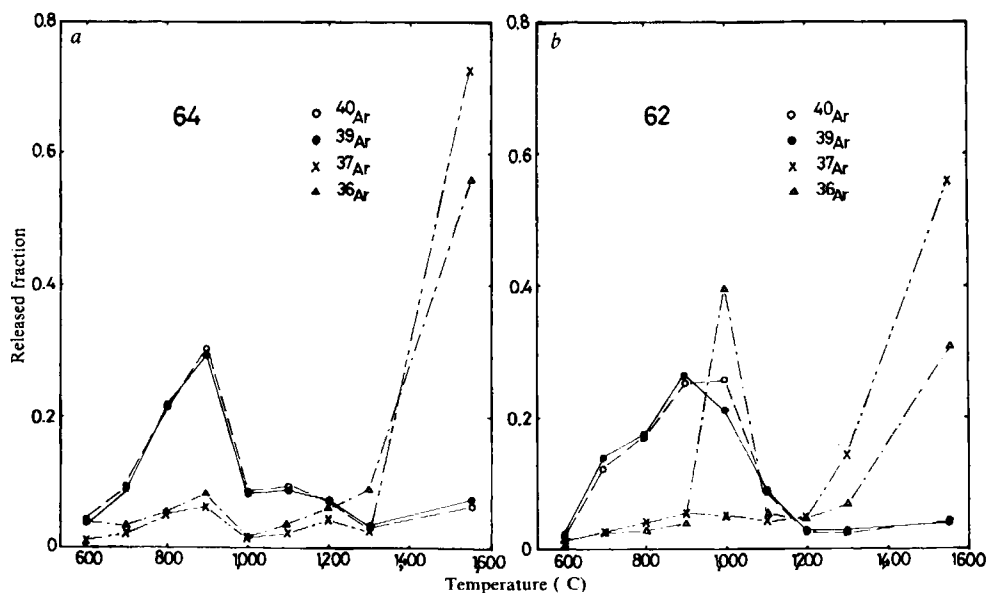
Since meteorites do not contain terrestrial-atmospheric components in general, such components, which are generally degassed at lower temperatures, should be incorporated secondarily. In the present samples, however, such old ages are observed in relatively high-temperature fractions. To explain this, some retentive phase is required. As a candidate, haematite

is proposed here which might have been changed from goethite during neutron irradiation. It has been reported that goethite changes into haematite at 165 °C for 19 days and at 135 °C for 125 days, respectively¹⁴. Since the maximum temperature during the neutron irradiation in JMTR has been estimated to be ~300 °C and the present samples were irradiated for 25 days, it is quite possible that goethite changed into haematite. Since samples were stacked in vacuum-sealed quartz vials, the desorbed atmospheric Ar would to some extent be trapped again in haematite during its formation.

Similar results were obtained for the other sample ALH-77288 of H6 type, whose original size was ~10 cm in diameter. The oxidized outer portion shows anomalously old ^{40}Ar - ^{39}Ar ages which exceed 5,000 Myr in the 800–900 °C fractions. No such phenomenon is observed for the fresh inner portion which has a plateau age of $4,497 \pm 40$ Myr (1,000–1,300 °C). The slight decrease in the degassed temperature compared with ALH-761 probably reflects both the experimental uncertainty of the temperature control and the difference of the thermal conductivity of the sample. The anomalously old ^{40}Ar - ^{39}Ar ages are also accompanied by the increase of ^{36}Ar for this sample. Hence the same explanation can be applied for the sample ALH-77288 as that for the sample ALH-761.

These results suggest that the weathering of Antarctic meteorites does not always decrease the ^{40}Ar - ^{39}Ar ages, but rather increases the ^{40}Ar - ^{39}Ar ages in some cases. Since such

Fig. 3 a, The release patterns of Ar for the sample ALH-761, 64. Note the good correlation between ^{40}Ar and ^{39}Ar . b, The release patterns of Ar for the sample ALH-761, 62. Note the anomalously high ^{36}Ar increase at 1,000 °C with slight deviation of ^{40}Ar from ^{39}Ar release pattern at the same temperature.



weathering is not restricted to Antarctic meteorites, we should be very careful in interpreting the ^{40}Ar – ^{39}Ar ages of meteorites which had been exposed for long time on the Earth's surface.

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Varied responses to subduction in Nankai Trough and Japan Trench forearcs

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Responses of sediment entering trenches range from accretion at the foot of the inner slope to subduction into the mantle, with inner trench slopes variously displaying uplift, subsidence, growth and retreat. Leg 87 is the latest of the DSDP voyages which have provided many clues to the behaviour of subduction zones, and was devoted to the study of subduction processes in two trenches off Japan. The Nankai Trough (Fig. 1) on the basis of very high quality reflection profiles^{1,2}, is accreting most or all its trench fill, which is being stripped off the underlying Shikoku Basin haemipelagites along a decollement near their contact (Fig. 2a). Because these seismic profiles define the large scale geometry so clearly, the objective of the Leg 87 drilling programme was to investigate the structural fabric and physical properties of sediments as they enter the subduction zone. Using these data which we now report, we hoped to deduce or constrain the stress conditions accompanying deformation.

The *Glomar Challenger* occupied two drill sites, Site 582 in the undeformed sediments of the trench floor and Site 583 in the stratigraphically equivalent but deformed sediments in the basal thrust sheet underlying the landward slope (Fig. 2a, b). Unfortunately our objectives were only partly achieved because of a series of mechanical problems and a typhoon. A deep hole originally intended to penetrate the basal thrust sheet (583E, F, G) was terminated just short of the fault, precluding the determination of changes in lithology, structure, and physical properties associated with the thrust. Nonetheless, drilling at Sites 582 and 583, together with the reference seismic profile, produced several noteworthy observations. Some of these arise from the absence of characteristics that we expected to encounter.

The reference seismic profile demonstrates that thrusts, rather than folds as interpreted from earlier drilling^{3,4}, underlie

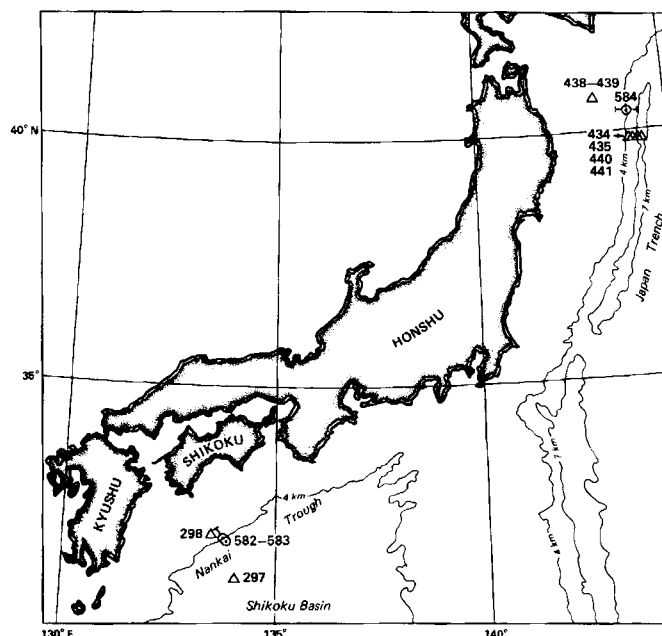


Fig. 1 Location map of DSDP Leg 87 Sites 582, 583 and 584, off Japan, together with location of earlier, related sites. The approximate locations of the two seismic profiles of Figs 2 and 3 are indicated.

the landward slope. The basal slabs, ~5 km wide, are bounded by thrusts with dips near 30° and show an arcward increase in internal deformation. Drilling results at Site 583 delineated an anticline over the nose of the basal thrust, but no significant folding was associated with the thrust at depths ≥ 300 m (Fig. 2b). This structural geometry fits the pattern of a bedding plane step thrust with a hanging-wall anticline developed over the top of a 30° ramp.

Small-scale structures at Site 583 are subtle and limited in diversity. A conjugate set of unhealed fractures dipping near 40° with well developed dip-slip grooves and striae was developed within 150 m of the projected fault surface in the deepest holes (Holes 583 F and G). We identify these fractures as compressional features based on the horizontal intersection lineation of the fracture pair which, from palaeomagnetic data, runs parallel to the trench, on the horizontal acute angle between the fractures, and on the concentration of fracturing near the thrust. A more steeply dipping system of faint dark bands occurs with the fractures and appears to be incipient narrow kink bands. No extensional structures, such as have been reported in the drill holes of several other inner trench slopes^{5,6} and at Site 584, were observed in the Nankai inner slope.

Tectonically-induced dewatering of the strata within the landward slope, anticipated from sampling of similar trench slopes⁷ and from experimental studies,⁸ was not observed. The porosity gradient of the trench-fill sediments at Site 582 corresponds to that of Site 583, as well as that of Site 298, drilled slightly upslope and along strike. Despite this lack of tectonically-induced dewatering, shear strengths at Site 583 are significantly higher than those at equivalent depths in Site 582, the reason for which awaits post-cruise investigation. The most pronounced example of dewatering and microdeformation was observed in the haemipelagites beneath the trench fill at Site 582, where small-scale normal faults are associated with steeply dipping 'dewatering' veins. This haemipelagite section at Site 582 also displays a marked reduction of porosity compared with that at Site 297, seaward of the trench, from <65% to ~45%. The near halving in thickness of this section required by such dewatering is observed on reflection profiles to occur beneath the southernmost (seaward) 3–4 km of the trench fill, which we assume to have supplied the causative overburden

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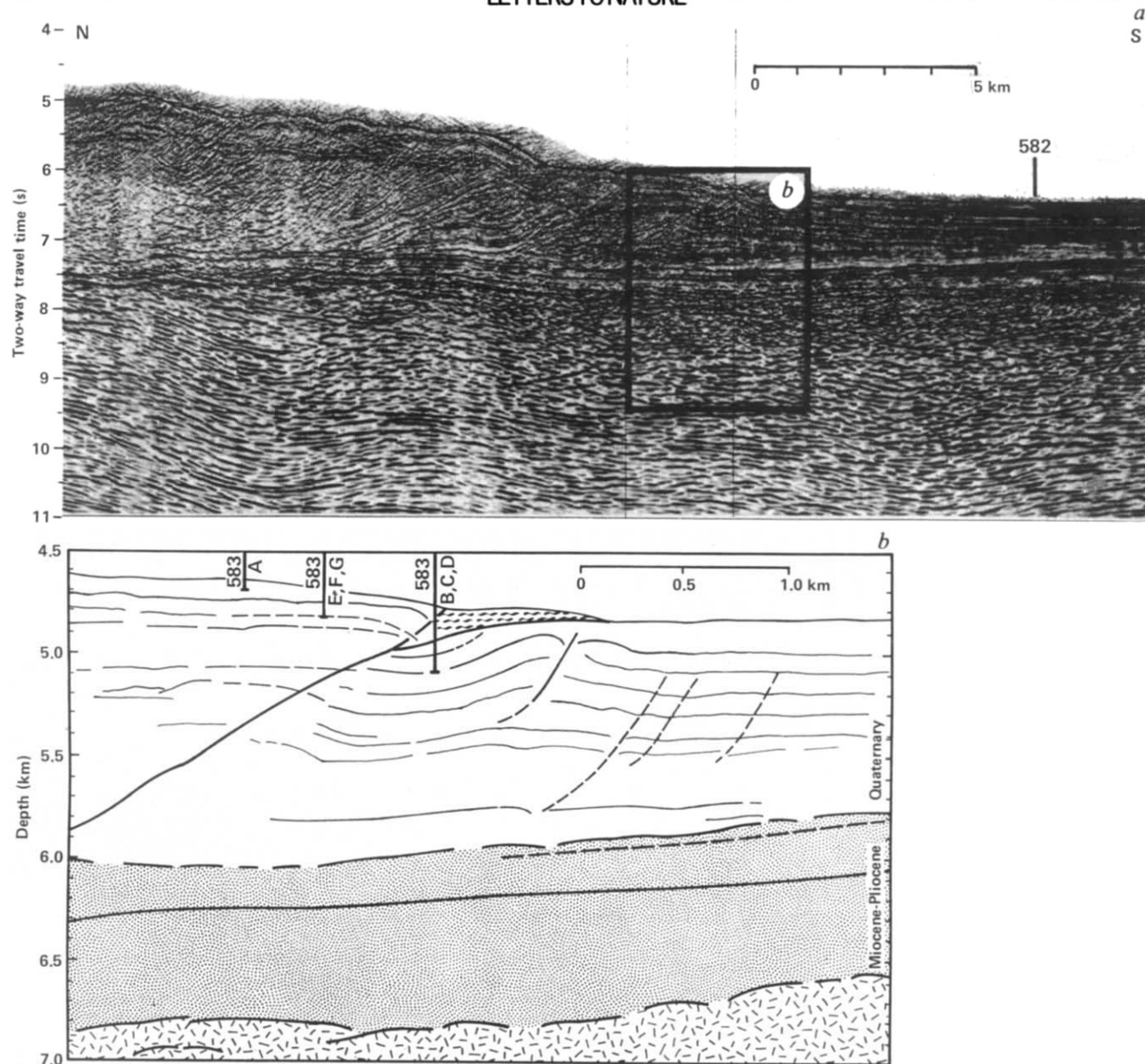


Fig. 2 Seismic time section (a) and enlargement of interpretive depth section (b) through Site 583 at the base of the inner slope of the Nankai Trough. The stippled pattern denotes the Shikoku Basin haemipelagic sediments and minor turbidites that underlie sediments ponded in the Nankai Trough. Hatching denotes oceanic crust. The wavy lines at the toe of the thrust indicate the area of possible chaotic, slumped sediment.

stress. High pore pressure, developed during dewatering, could lower the effective confining pressure in the haemipelagites; thus the extension associated with flexure of the descending plate could be sufficient to cause the observed normal faulting.

The south-west Japan arc, which includes the Nankai Trough in part, is the site of frequent, very large earthquakes. Seismological studies have inferred subduction rates near the drilling sites in excess of 4 cm yr^{-1} (refs 9, 10). It is surprising, therefore, that the sedimentation rates encountered in the trench at Site 582, combined with the trench-fill geometry supplied by the reflection profiles, imply a subduction rate, based on generally assumed steady-state conditions¹¹, of $< 2 \text{ cm yr}^{-1}$. This discrepancy between geological and seismological evidence seems to be real, but is as yet unexplained.

The response to a low rate of convergence in the Nankai Trough appears on reflection profiles to be well-organized selective accretion of the thick trench-fill clastic deposits. The deformation, although mild, appears to be compressional on all scales. No highly disrupted samples, or *mélange* were recovered, although a zone of chaotic, slumped and overridden

sediment is inferred beneath the toe of the thrust from mass-balance calculations across this structure (Fig. 2). Preliminary calculations suggest that of the 2 cm yr^{-1} slip rate, only a quarter or less is being accommodated on the basal thrust and across a proto-thrust zone to the south. Continued arcward thickening of the thrust slabs, together with a degeneration of acoustic coherence, and the progressive downward tilting of sediments in the one slope basin defined on the reference seismic profile, support the idea that deformation is distributed across the inner slope, although it is probably most intense near the slope base.

A more complicated history of plate convergence along the northeast Japan Arc had previously been outlined by deep-sea drilling and extensive geophysical surveying¹²⁻¹⁴. Late Cretaceous and early Palaeogene tectonics produced, or were associated with, uplift that created the landmass of Oyashio in the outer forearc. Subsequent large-scale subsidence of the forearc, from latest Oligocene into the latest Pliocene, is attributed to subduction-induced tectonic erosion. The post-earliest Pleistocene response to convergence seems once again to be uplift and accretion.

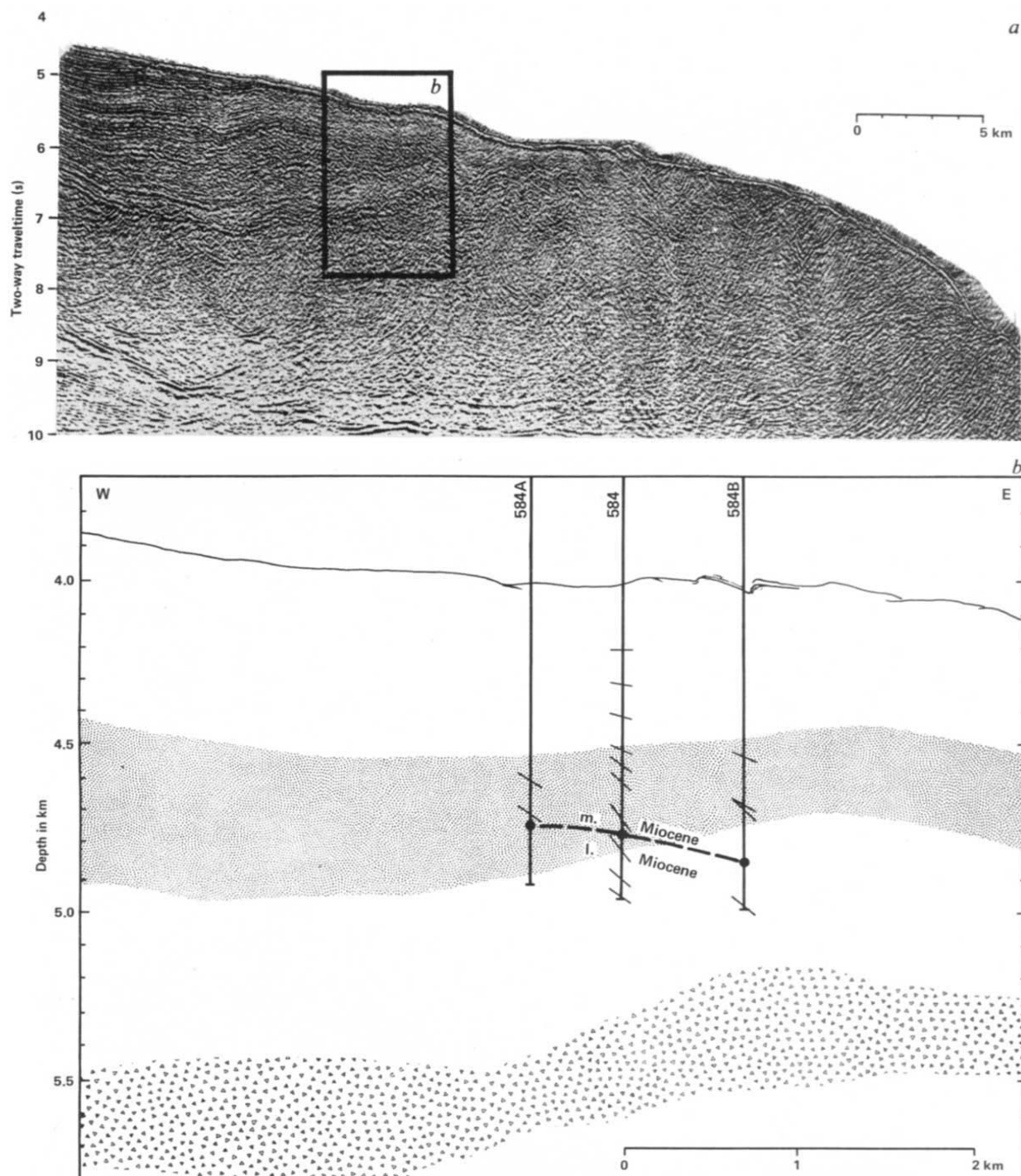


Fig. 3 Seismic time section (*a*) and enlargement (*b*) of the interpretive depth section through Site 584, on the midslope terrace of the Japan Trench. Dips of strata in cores are shown for each hole and indicate the contrast with the attitudes of the biostratigraphical horizons and with the upper seismic reflecting horizon (stippled pattern). The triangular pattern denotes the deeper reflectors presumed to mark the Cretaceous to Palaeogene rocks beneath the trench slope.

DSDP Leg 87 included one drill site (Site 584), located on or slightly landward of the midslope terrace, where an early Palaeogene unconformity appeared to come close enough to the surface to be sampled (Fig. 3). Objectives at this site included the calibration of the Neogene subsidence history on the outer edge of the forearc, a test of the nature of the deep reflection presumed to mark the Cretaceous unconformity, and the understanding of tectonic processes affecting slope sediments.

Previous drilling¹² led us to expect a thick late Neogene sequence of deep-water muds, shallowing downward through most of the Miocene into a terrigenous sequence eroded from the Oyashio landmass. The structure suggested by a processed

seismic profile, although not well resolved, appeared simple, with nearly horizontal strata overlying a local relative uplift of the unconformity (Fig. 3).

Because of persistent caving near 900 m in the three holes drilled at Site 584, the deep unconformity could not be reached, but the Lower Pliocene to lowermost middle Miocene strata which were recovered delineated an unexpected structural geometry. Bedding dips increase downhole, until below 600 m strata dip 40–70°; palaeomagnetic measurements indicate dips to the east. Beginning gradually in the Lower Pliocene near 220 m, the diatomaceous muds exhibit tilted beds, dewatering veins, and normal microfaults at high angles to bedding. These are often cut by moderately dipping shears with much larger

offsets, all of which are healed. Relative displacement and rotation define a progressive sequence of dewatering followed by microfaulting and that, in turn, by large-scale tilting.

Two offset holes, drilled in an attempt to avoid the highly-fractured zone near 900 m, demonstrated that, over a 1 km cross-dip spread, biostratigraphical horizons dropped only 100 m eastward, far less than would be required by an unbroken sequence of the observed dips. Numerous normal faults, possible with westerly dips, must thus offset the section. These may be related to westerly dipping normal faults observed upslope near Sites 438 and 439 (ref. 12). Structural activity at Site 584 proceeded progressively, from within the Miocene at least into the early Pliocene.

Although dewatering and fracturing were recognized in the previously drilled sites, the large-scale tilting was not obvious. Accordingly, tectonically-induced dewatering and resultant fracturing were primary considerations in the Leg 56-57 report¹⁴. Such a process may occur near Site 584, but alone it is not sufficient to produce large tilted blocks and normal faults, structures that strongly suggest large-scale extension. If the style of deformation is similar to that at Sites 438 and 439, but more intense, it is reminiscent of shattered fault blocks tilted into normal-fault surfaces, features observed in the US Basin and Range and on passive margins¹⁵ (although with oceanward rather than landward rotation). Should similar features form the Japan Trench margin, a decollement is required against which the steeply dipping beds above the subhorizontal basement could be resolved.

Extensional tectonism in the forearc is no longer a novel observation^{5,6}, but the apparent magnitude of this displacement near Site 584 is new. How this extension relates to the subsidence history of the forearc and to the transition in regional stress regime as stress perpendicular to the arc changes from extensional to compressive¹⁶, remains a major problem. Attempts to piece our observations into previously existing models and to relate them to more fundamental processes reveal a wide diversity of opinion among shipboard scientists.

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Basaltic systems and a corresponding states equation for crystal growth rates

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Rates of crystal growth are a major influence on the processes of igneous rock crystallization but relatively few measurements are available. Here I examine temperature variations of growth rate for basaltic minerals in terms of an empirical relationship proposed for small organic molecules^{1,2} and show that this describes equally well rates of growth in the basaltic system. These results suggest useful applications of the relationship in studies of grain growth during cooling and also in the extrapolation of experimental data.

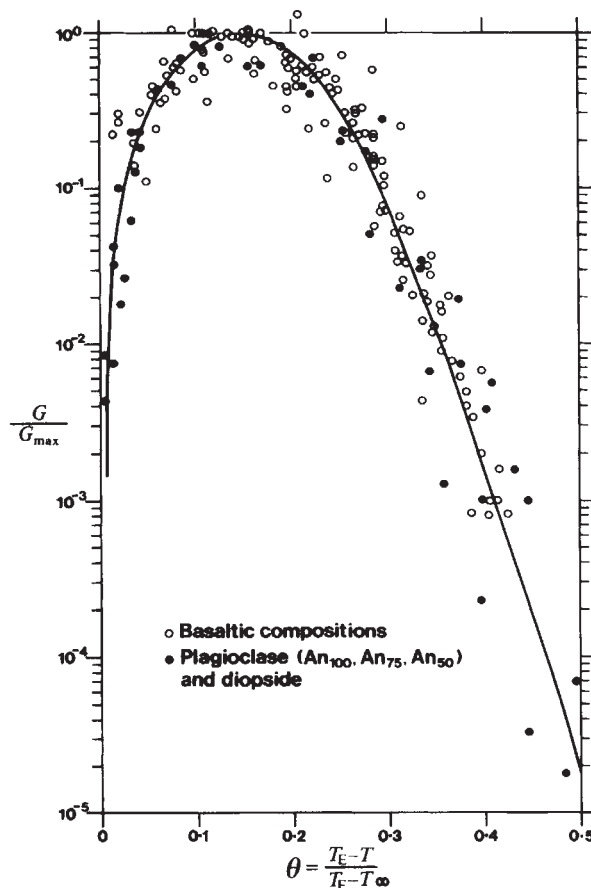


Fig. 1 Growth rate (G) as fraction of peak growth rate ($G_{\max}$) against a reduced temperature function $\theta = (T_E - T)/(T_E - T_{\infty})$; see text. Points plotted are taken from published growth rate curves for lunar basaltic compositions³⁻⁹, a glass-rich (howardite) meteorite¹⁰, diopside^{11,12} and plagioclase feldspar¹²⁻¹⁴. The curve is based on data from small organic molecules^{1,2}.

An approach towards a general description of crystallization behaviour has been suggested^{1,2} which is based on the experimental results from various small organic molecules. A growth rate ratio is related to a reduced temperature function θ :

$$\log (G/G_{\max}) = f(\theta) \quad (1)$$

in which $\theta = (T_E - T)/(T_E - T_{\infty})$ and where G is the growth rate at temperature T , $G_{\max}$ is the maximum growth rate and T_E the equilibrium temperature. T_{∞} is considered as the kinetically limiting value of the glass transition temperature (T_g) at infinite time. At T_E and T_{∞} the growth rate tends to zero and the maximum growth rate ($\log G/G_{\max} = 0$) is at $\theta = 0.14$ (see Fig. 1). This relationship, using growth rate and temperature difference ratios and termed a corresponding states equation^{1,2}, does not assume any specific nucleation or transport mechanism but does require a knowledge of T_{∞} . Before discussing applications to basaltic minerals some features of the relationship require comment.

The corresponding states equation (CSE) may be combined with the standard Arrhenius growth rate equation $G = G_0 \exp(-Q/RT)$ by the use of a fixed ratio between the growth rate at $\theta = 0.14$ (the maximum growth rate) and that at any other temperature. Taking a temperature within the region where growth rate decreases exponentially with temperature, where, say $\theta = 0.43$, then $G_{\theta} = 6.249 \times 10^{-4} G_{\max}$. The temperature function T_{θ} may be written as $T_{\theta} = T_E - 0.43(T_E - T_{\infty})$ leading to

$$G_{\max} = (G_{\theta}/6.249 \times 10^{-4}) \exp \{-Q/R[T_E - 0.43(T_E - T_{\infty})]\} \quad (2)$$

Practical use of equation (1) requires the evaluation of T_∞ in order to calculate θ for any given temperature. Different methods may be used depending on the available data. The first method requires only experimental values for T_E and Q , and is based on the form of the function of $f(\theta)$; two standard points are considered within the Arrhenius region of the CSE curve, for example, $\theta_1 = 0.430$, $\log(G/G_{\max})_1 = -3.2042$, and $\theta_2 = 0.525$, $\log(G/G_{\max})_2 = -5.4654$. Then

$$\log(G_2/G_1) = (Q/2.3026R)(1/T_1 - 1/T_2) \quad (3)$$

may be rewritten

$$10.3466/Q = (1/T_1 - 1/T_2) \quad (4)$$

and from the above values of θ_1 and θ_2 equation (5) is obtained

$$\Delta T_1/\Delta T_2 = 0.81905 \quad (5)$$

where $\Delta T_1 = T_E - T_1$ and $\Delta T_2 = T_E - T_2$.

Therefore, the two temperatures T_1 and T_2 , which simultaneously satisfy equations (4) and (5), may be found by iteration, then

$$T_\infty = T_E - (\Delta T_1/0.430) = T_E - (\Delta T_2/0.525) \quad (6)$$

Also, T_∞ is given by

$$T_\infty = (0.14T_E - \Delta T_m)/0.14 \quad (7)$$

where ΔT_m is the undercooling at $G_{\max}$.

Data plotted on Fig. 1 for comparison with the CSE curve were taken from published measurements of growth rate for lunar basaltic compositions³⁻⁹, a glass-rich (howardite) meteorite¹⁰, diopside^{11,12} and plagioclase feldspar¹²⁻¹⁴. These experimental results cover about five orders of magnitude of growth rate and undercooling values up to $\sim 650^\circ\text{C}$. Maximum growth rates vary by almost three orders of magnitude and the apparent activation energy for growth, Q , is in the range 60–124 kcal mol⁻¹. Materials used in the construction of the (CSE) curve² have melting temperatures which vary from 1 to 199 °C and apparent activation energies for growth which are much below the basaltic range although the range of maximum growth rates is similar for both sets of data.

To plot the $G/G_{\max}$ ratios for the basaltic data onto Fig. 1 the characteristic value of T_∞ is required for each growth rate curve. This may be derived from Q and ΔT_m (estimated from a $\log G$ versus $1/T$ plot) and by setting these parameters in equations (4)–(6) and equation (7) respectively; the mean value of T_∞ is then used to calculate θ for the temperature at each data point.

The root mean square difference (r.m.s.d.) between the basaltic data points on Fig. 1 and the CSE curve is given by $(\sum_1^n (\log G_B - \log G_C)^2/n)^{0.5}$ where n is the number of observations and G_B and G_C are the $G/G_{\max}$ ratios of basaltic data and the CSE curve respectively at a given θ . The data ($n = 179$) from 18 growth rate curves yields a r.m.s.d. of 0.31. This value is less than the r.m.s.d. of 0.413 (with $n = 89$) for the organic molecule data which define the CSE curve and indicates that the basaltic growth rates lie within a factor of 2.15 of those given by the CSE curve.

These results show that for basaltic compositions and minerals, as well as for a wide variety of pure minerals crystallizing from their melts, the CSE relationship provides a relatively close approximation to crystallization behaviour, although more experimental data are required to test the relationship for natural silicate minerals in general. As pointed out by Magill *et al.*^{1,2}, the relationship not only allows the testing of crystallization data for internal consistency but also enables extrapolation of limited data. A particularly valuable feature of the relationship is the ability to define the whole temperature dependence of growth rate from T_E and T_∞ ; it has been found useful in this respect for modelling the kinetics of solidification and grain growth during cooling of basic rocks.

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Overestimate of stalagmitic calcite ESR dates due to laboratory heating

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In the use of ESR for dating stalagmitic calcite from palaeolithic caves it has recently been reported that to obtain reliable ages the samples should be heated before measurement, for example, for 24 h at 170–190 °C. Specifically this procedure has been used by Yokoyama *et al.*¹ for samples from the lowest stalagmitic floor in Caune de l'Arago, the site of the Tautavel man in southern France, and an age of ~ 700 kyr obtained. However, the results presented here indicate that straight-line extrapolation involved in finding the age is not valid if this heating procedure is used and that its use leads to a substantial overestimate of the age, by nearly a factor of two in the application mentioned.

To determine the age of a sample by ESR, its sensitivity to radiation is measured by artificially irradiating it (in this case with γ radiation from a ¹³⁷Cs source). The signal intensity is plotted as a function of applied dose, and the known values are extrapolated to zero signal (Fig. 1). The x-intercept gives the value of the γ -equivalent archaeological dose (ED) acquired by the sample over time. One then determines independently the intensity of natural radiation both from radioactivity within the samples and from radioactivity in the surrounding sediment at the sample site; the age is simply the ED divided by the annual dose. (Note that if the dose contributed by radionuclides within the sample is a significant portion of the total dose, a correction should be made for disequilibrium in the uranium decay series to allow for 'grow-in' of ²³⁰Th.)

The room-temperature ESR spectrum of stalagmitic calcite generally has three peaks attributable to radiation-induced defects in the calcite, with g -values of h_1 , 2.007; h_2 , 2.004 and h_3 , 2.001 (present work). Normally other peaks attributable to the presence of Mn²⁺ are also present in the sample (Fig. 2). To be useful in dating, the ESR peak must grow linearly with applied radiation. Both h_2 and h_3 grow when the sample is irradiated, but h_2 is unstable, and no error appears to be introduced into the present results by neglecting it. Thus the intensity of h_3 has been used to obtain the sample age.

The age range of stalagmites of archaeological interest is $\sim 10^4$ – 10^6 yr. In 'old' calcites, those in the upper part of this range, the very sharp peak h_1 appears to be insensitive to the artificial radiation. However, on heating such a sample, h_3 shrinks and h_1 grows. Recently it has been suggested² that the spectrum of calcite reflects the existence of three types of defect which are related in the sense that electrons can transfer from the least stable to the most stable with time, a process accelerated by heating. The defect corresponding to h_2 is the least stable; the next most stable is that corresponding to h_3 ; and h_1

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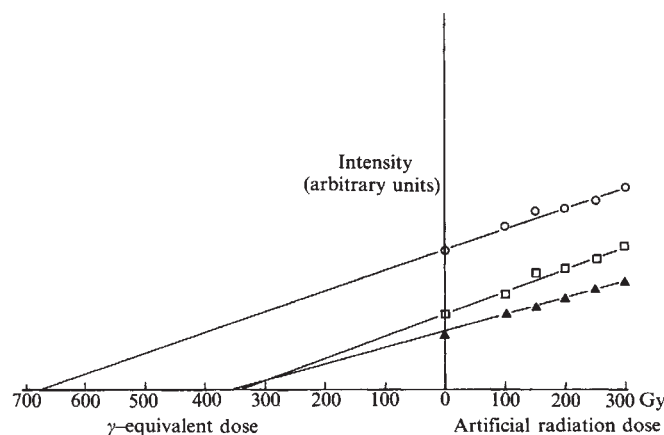


Fig. 1 Results from treating 'old' calcite (lowest level, Caune de l'Arago, laboratory reference, 211a29). Intensities (in arbitrary units) of: ○, h_1 , after annealing for 24 h at 184 °C; ▲, h_3 , before heating; □, change in h_1 due to heating.

is by a wide margin the most stable. Furthermore, it has been suggested that annealing experiments showed h_3 to be insufficiently stable to be used for dating calcites as old as 500 kyr.

The assumption was then made that the defect corresponding to peak h_1 cannot be populated directly through irradiation (since the intensity of h_1 does not change when the sample is irradiated artificially), and hence the initial intensity of h_1 (that found in the sample before heating or artificially irradiating) must result from the transfer of electrons from h_2 and h_3 to h_1 over time. In that case correct ages can only be found by assessing the sum of all calcite peaks. This is most easily done by heating the sample and measuring the final intensity of h_1 . Figure 1 shows the results of a typical experiment using a sample from the lowest level at Caune de l'Arago. Extrapolation of the intensity of h_3 gives an ED approximately half that found by extrapolating the intensity of h_1 after heating. Since the annual dose to be inserted in the age equation is the same in both cases, using the ED of the annealed samples gives an age which is nearly twice that obtained from the usual interpretation of the ESR data. These dates^{1,2} are also much older than found by thermoluminescence (TL), and the ED depends on annealing temperature³.

There is no doubt that the basic structure of the model in ref. 2 is reasonable. We have had no difficulty in reproducing the results on old calcite samples that were used to derive the model. Furthermore, we have plotted the increase of h_1 on heating (as well as the final intensity), and as seen in Fig. 1, extrapolation of the increase gives the same ED as found using h_3 , a strong indication that there is at least proportional, and possibly quantitative transfer from h_3 to h_1 . Of course, this does not conclusively demonstrate that the initial intensity of h_1 must arise solely from similar transfer of electrons from h_3 over time. Unfortunately, we cannot drain the defects corresponding to h_1 and then reirradiate the sample to reproduce the initial growth. Even heating for several hours at 350 °C merely reduces h_1 and does not eliminate it. Heating the sample above 350 °C gives a new ESR line, broad enough to mask the h_1 signal.

To test the model further, we looked at calcites from the 'young' end of the age range, ~10 kyr. In young samples one would expect to find little, if any, initial h_1 intensity. One would also expect that the extrapolations of h_1 (after heating) and h_3 would be in better agreement, since even by the most restrictive calculations, the lifetime² of h_3 is 200 kyr, well above 10 kyr.

The results for a typical young calcite are shown in Fig. 3. This sample has been dated by U series disequilibrium at ~20 kyr BP. The annual dose, both internal and external, is 1.7×10^{-3} Gy yr⁻¹. We would thus anticipate an ED of ~34 Gy for this sample. The extrapolation of h_3 gives 38 ± 12 Gy. A similar result was found by Hennig (personal communication). However, the behaviour of h_1 has changed dramatically. If we

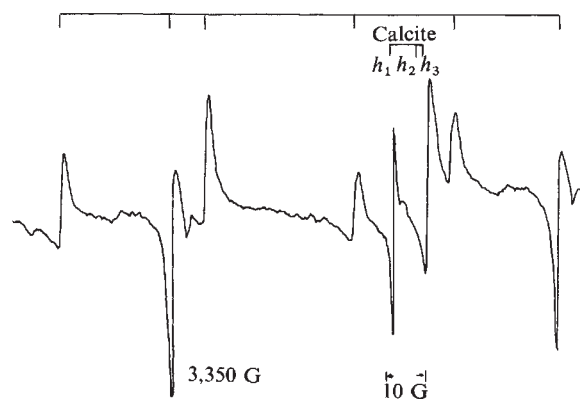


Fig. 2 ESR spectrum of typical calcite showing peaks due to trapped electrons in calcite and peaks due to presence of trace amount of Mn^{2+} . Modulation amplitude: 0.40 G; magnetic field increased from left to right. Because of its instability, the h_2 peak is only seen in artificially irradiated samples.

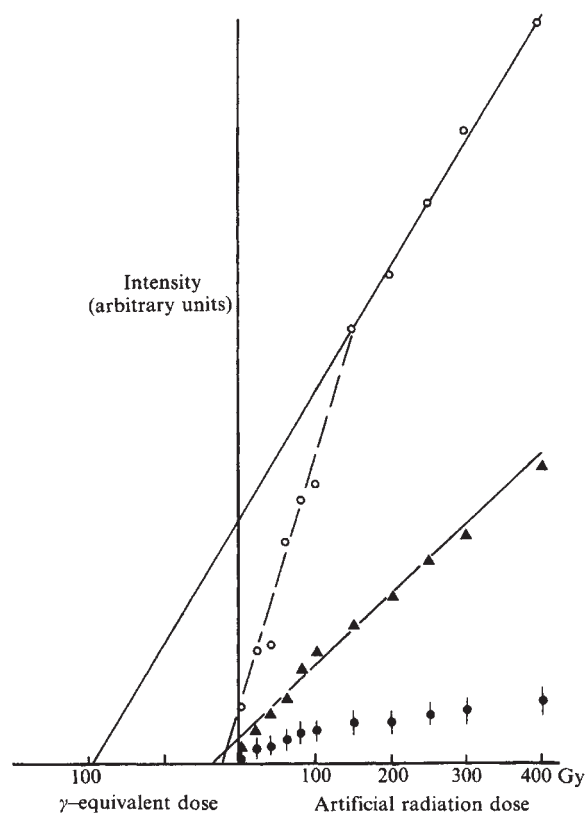


Fig. 3 Results from treating 'young' calcite (Trou Ballou, Belgium; laboratory reference, 227a1). Intensities (in arbitrary units) of: ○, h_1 after heating for 24 h at 180 °C; ▲, h_3 before heating; ●, h_1 before heating. Size of points represents 1σ uncertainties (except for h_1 before heating, where error bars are drawn).

look only at the values for h_1 found for heated samples that have had an artificial dose of at least 150 Gy (the order of magnitude of the natural dose of an old calcite) we find that they extrapolate to 90 Gy, in as poor agreement with the h_3 value (38 Gy) as for old calcite (see Fig. 1). The explanation is found by looking at the height of h_1 after heating for samples with comparatively small doses on them. The rate of increase in intensity of h_1 (after heating) with artificial irradiation is much less for doses ≥ 150 Gy than it is in the range 0–150 Gy.

In evaluating linearity (or lack of it), one must always consider the uncertainties in the data points. Peak h_1 (after heating) is sharp, well-resolved, and roughly three times the intensity of h_3 . Furthermore, determination of the intensity of h_3 is complicated by the presence of shoulders on both sides. The scale

chosen for Fig. 3 reflects the relative uncertainties by plotting h_1 (heated) on a scale 2.5 times that of h_3 , such that the sizes of the points in each case represent 1σ . Analysis of the data for fit to a straight line shows that for h_1 the χ^2 test gives a vanishingly small probability of a 'good' fit, and further that of the 11 points, 6 lie more than 2σ from the best line. In contrast, the points for h_3 fit the line at $\sim 20\%$ probability, with no points more than 2σ from the line. This analysis does not preclude the possibility of curvature in the h_3 points. It merely says that the curvature in h_1 (heated) is statistically significant, relative to the known uncertainties in measurement, and the observed reproducibility of the results. When the h_1 (heated) data points are subdivided into two sets (shown as two straight lines in Fig. 3), both sets can be fitted to straight lines, although the point at 40 Gy must be considered questionable. Note that each data point represents a different aliquot of powdered stalagmite. For a given artificial radiation dose a sample of about 0.25 g was irradiated and then divided in half (one half left unheated: the other heated).

Furthermore, examination of the intensity of h_1 before heating shows that it does in fact respond to radiation in young calcites. The size of h_1 is very small, so the uncertainties in the points shown in Fig. 3 are too large to allow one to determine the shape of this growth curve with any accuracy. However, there is real growth over the range of radiation dosages used, but most of this occurs in the first 200 Gy, again the region that cannot be seen in older samples.

These results suggest that the behaviour of h_1 is very complex. Its intensity increases with irradiation up to a certain point, but even after it can no longer be increased by irradiation, it can be increased by transfer due to heating. There are several theoretical models of the h_1 defect that could be suggested for this behaviour.

In old calcites the increase in h_1 intensity on heating can be explained solely by assuming transfer from h_3 , but in young calcites we have found that the initial growth in h_1 is more rapid than can be derived from transfer from h_3 alone. A possible explanation for this observation is that there exist one or more other peaks representing electrons that can also be transferred to h_1 , but that these defects saturate much more readily than does the defect corresponding to h_3 , and therefore transfer from them increases the rate of growth of h_1 only at low dosages. Two possible peaks of this sort were found in one young calcite, which happened to contain no Mn^{2+} . The peaks appear at g values 2.012 and 1.998, essentially the same as the two peaks which flank the calcite peaks in Fig. 2. However, unlike the peaks in Fig. 2, these peaks disappear in the heated young samples. While these peaks do not appear to saturate completely over the 400 Gy range, their maximum rate of growth occurs in the first 100 Gy. (Other than possibly masking calcite peaks, the presence or absence of Mn^{2+} does not appear to affect the behaviour of the ESR signal.)

While these results show that one cannot use extrapolation of heated samples to obtain ESR dates for calcite, they do not address the major concern that the h_3 peak is insufficiently stable to be used for dating (that is, that the signal has decayed with time so the age obtained is an underestimate). However, there is both direct and indirect evidence, on this point. Isothermal annealing experiments on calcite have yielded a mean lifetime for the ESR signal of $\sim 10^8$ yr at ambient temperatures⁴. In addition we can compare ESR EDs and TL EDs. Over the full range of samples used in this work, the extrapolation of h_3 gives an ED in excellent agreement (within experimental errors) with that found by TL, using the peak at around 280 °C. This does not necessarily mean that the two signals arise from the same defect. While both ESR and TL measure similar phenomena (trapped electrons), there is no requirement that every ESR peak correspond to a TL peak, or vice versa. It does indicate that whether or not the defects are identical, the ESR h_3 peak is no less stable than the 280 °C TL peak. Evidence has been presented that the mean life time of the TL peak is in excess^{5,6} of 10^6 yr for an ambient temperature of 10 °C.

Thus I conclude that the initial intensity of h_1 is partly due to direct filling of the traps during irradiation of the material over time, plus perhaps a contribution from peaks other than h_3 , and h_1 , no matter how stable, cannot presently be considered a suitable signal for dating purposes.

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Reliability of thermoluminescence dating of stalagmitic calcite

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The dating of stalagmitic calcite at the early hominid site of Caune de l'Arago, France, by the ESR technique of Yokoyama *et al.*¹, has yielded ages for the underlying floor of $\sim 7 \times 10^5$ yr. These are in serious disagreement with the chronology obtained by thermoluminescence (TL)², which indicates a date of around 3×10^5 yr. The discrepancy has been attributed³ to instability of the TL 280 °C dating signal, for which a mean life of 2×10^5 yr was proposed. Here the arguments on which this claim is based are criticized, and evidence is presented that corrections to TL age measurements incurred by signal fading are, even for the oldest stalagmites from the site, probably $<15\%$. The conflict between the TL and ESR chronologies at Caune de l'Arago is explicable in terms of the ESR results reported by Skinner⁴.

Three lines of evidence have been advanced³ to support a mean life for the 280 °C TL peak of 2×10^5 yr: (1) TL decay rates, calculated by an extended initial rise method, in the temperature range 220–340 °C, (2) annealing rates of the h_3 ESR signal between 155 and 200 °C, and (3) indications in old samples of fading in the h_3 ESR signal by comparison with the annealed h_1 signal. In the present work, phosphorescence decay rates in a stalagmite from Caune de l'Arago (laboratory reference 211a26) were measured at six temperatures between 175 and 236 °C. The error limits on the mean life measurements are $\pm 9\%$ or better, and the temperature scale, calibrated against TL peak temperatures of CaF_2 (MBLE) and CaSO_4 : Mn at low heating rates⁵, is considered accurate within ± 1 °C. The results are compared in Fig. 1 with the TL decay rates presented by Yokoyama *et al.*³. There is fairly close agreement between these two sets of data, the small discrepancy probably being due to a systematic temperature difference of ~ 4 °C. The phosphorescence measurements are well fitted by a straight-line in the Arrhenius plot, the slope of which corresponds to an activation energy, $E = 1.56 \pm 0.08$ eV. Extrapolation of the fitted line to 15 °C yields a mean life of 10^6 (± 3) yr. From their TL data, Yokoyama *et al.*³ obtained a mean life at 15 °C of 2.0×10^5 yr, using an extrapolation with $E = 1.44$ eV. A line of this slope gives a poor fit to our phosphorescence measurements. On the other hand, it appears that the data of ref. 3 are equally well fitted by a line with the same slope as that fitted to the phosphorescence data (for example, line *d* in Fig. 1). I conclude from this that the TL data presented in ref. 3 do not exclude

extrapolations which yield mean life values at 15 °C of 10^6 yr or more. (Note that the extrapolated mean life of 10^6 (≈ 3) yr is not necessarily in conflict with the value of 3×10^7 yr found by Wintle⁶, since her measurements were made (with a Corning 4-96 filter) on the green and blue wavelength emissions of calcite, while both sets of TL data discussed here were, for reasons given elsewhere⁷, based on observations in the blue part of the spectrum alone.)

Also shown in Fig. 1 are the decay rates, presented in ref. 3, of the h_3 ESR signal in the temperature range 155–200 °C. These data differ greatly from our phosphorescence decay measurements on the 280 °C TL peak. For instance, in ref. 3 the mean life of the h_3 ESR signal at 200.5 °C is given as 2,640 s, whereas the 280 °C TL peak has a mean life of only 730 s at the same temperature. Moreover, the temperature dependence of the h_3 decay rates, corresponding to a trap depth, $E =$

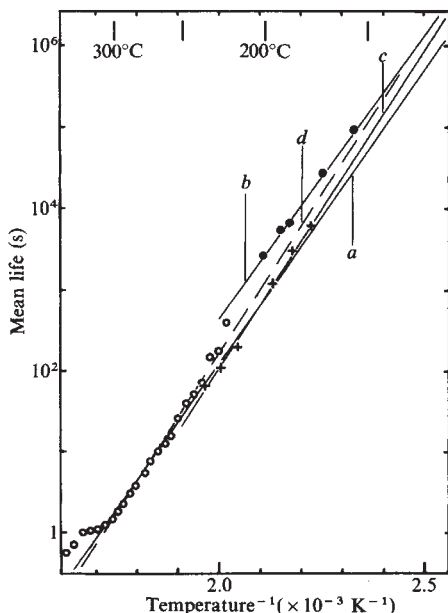
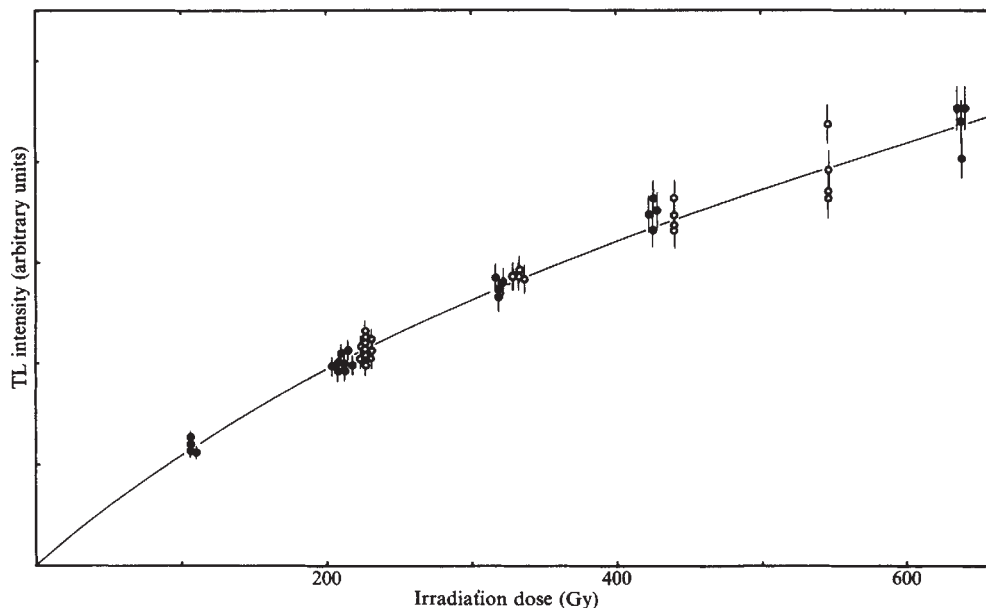


Fig. 1 Arrhenius plot showing decay rates of the 280 °C TL peak (○), and h_3 ESR signal annealing rates (●) (both from ref. 3), together with the phosphorescence TL decay measurements (crosses) presented here. The following straight lines are drawn: a, extrapolation of TL data as in ref. 3 ($E = 1.44$ eV); b, extrapolation of ESR data as in ref. 3 ($E = 1.37$ eV); c, least-squares fit to phosphorescence measurements ($E = 1.56$ eV); d, fit by eye to ref. 3 TL data with line of slope $E = 1.56$ eV.

Fig. 2 Equivalent dose determination using the 350 °C TL peak of a sample of stalagmitic calcite (211f1) from the lowest floor at Caune de l'Arago. The first glow (○) and second glow (●) growth curves of this TL signal with β irradiation are compared. The first glow data have been shifted along the dose-axis by an amount, D , and its height multiplied by a factor, F , (which represents the effect of TL sensitivity and opacity changes on first heating). These two parameters were varied to find the optimum match between the two data sets, as assessed by fitting the data to a single curve. The best match configuration for this sample, having $D = 216$ Gy and $F = 1.20$, is shown together with the fitted curve. The 350 °C TL data thus indicate an archaeological equivalent dose, $ED = 216^{+30}_{-30}$ Gy.



1.37 eV, is removed by more than 2 s.d. from that of the 280 °C TL peak ($E = 1.56 \pm 0.08$ eV). Therefore, on the basis of the presented data, there is no justification for identifying the h_3 ESR signal as resulting from the same trapped electrons as are responsible for the 280 °C TL peak. Hence, it cannot be claimed that measurements on one have implications for the other.

The third piece of evidence³ for instability of the 280 °C TL peak involves the ratio between the equivalent doses (ED) of the h_3 and annealed h_1 ESR signals ($P = ED_3/ED_1$). The argument assumes, first that P represents a fading factor for the h_3 signal relative to the annealed h_1 signal, and second, that the h_3 signal and the 280 °C TL peak arise from the same set of trapped electrons. Concerning the first assumption, in an accompanying paper Skinner⁴ demonstrates that the non-equality of ED_3 and ED_1 results not (necessarily) from fading of the h_3 signal, but from a failure to allow for the nonlinear growth of the annealed h_1 signal. The second assumption is, as argued above, not validated.

The long-term stability of the 280 °C TL peak is supported by the results of two experiments designed to measure directly signal losses in stalagmites from Caune de l'Arago. In the first, ED determinations were made using the 350 °C peak and compared with measurements on the 280 °C dating signal. (The higher temperature peak is assumed to be stable.) The second method made use of the plateau test⁸.

The 280 °C TL signal is used for dating in preference to the 350 °C peak because, while the former increases linearly with radiation exposure over a large dose range (up to at least 1 kGy), the higher temperature signal grows in a nonlinear fashion (Fig. 2). It is possible to determine EDs with the 350 °C signal, however, by the method illustrated in Fig. 2. The results of measurements performed on three stalagmites from the lowest

Table 1 Comparison of ED determinations on samples of stalagmitic calcite using the 280 °C and 350 °C TL peaks

Sample	ED(280 °C) (Gy)	ED(350 °C) (Gy)	Ratio ED(280 °C)/ED(350 °C)
211f1	199 ± 17	216^{+42}_{-30}	$0.92^{+0.15}_{-0.20}$
211f2	194 ± 22	223^{+70}_{-50}	$0.87^{+0.22}_{-0.29}$
211f3	155 ± 16	188^{+48}_{-35}	$0.82^{+0.17}_{-0.22}$
217a1	137 ± 13	128 ± 25	1.07 ± 0.23

Samples 211f1, f2 and f3, taken from the oldest formations at Caune de l'Arago, are $\sim 3.5 \times 10^5$ yr old. Sample 217a1, a young stalagmite which was given a known artificial irradiation (total dose 123 ± 8 Gy), provides a check on the method of ED measurement.

floor at Caune de l'Arago are compared in Table 1 with the corresponding EDs determined from the 280 °C peak. It is seen that the degree of fading of the TL 280 °C peak is confined by these data to the range 0–30%. This is in sharp disagreement with the values of 68–73% proposed in ref. 3 for the comparable samples, YC62e, 9UL8 and YCP17.

In the plateau test⁸, the natural material is given an additional artificial irradiation equal to the ancient dose, and the shape of its glow curve is compared with that of the natural glow curve. Five stalagmites from the lowest floor at Caune de l'Arago (211e7, e8, f1, f2 and f3) and one from an intermediate floor (211e3) were so tested. It was found that in none of these did the ratio of TL intensities, natural/(natural+artificial), increase by >7% over the 40 °C temperature interval centred on the 280 °C peak position. It was also found that the constancy of this ratio is not maintained when one or other of the glow curves has been partially drained by prolonged heating at an elevated temperature. Over the same 40 °C interval, a variation in the glow curve ratio of $18 \pm 3\%$ accompanied a 33% loss of TL at the 280 °C peak, while a 40% drainage caused variations in the range 29–41%. From these observations it is concluded

that the 280 °C TL peak of the oldest stalagmites from Caune de l'Arago has probably not faded in antiquity by more than 15%. From the age of these materials ($\sim 3.5 \times 10^5$ yr, as indicated by the preliminary results given in ref. 2), a lower limit of $\sim 1.0 \times 10^6$ yr may be calculated for the mean life of the dating signal. This is consistent with the extrapolated value at 15 °C gained from the phosphorescence measurements.

Samples were checked for anomalous fading⁹, but showed no measurable (within $\pm 3\%$) loss of γ -induced TL during 8 months of storage.

No valid basis has, therefore, been found for the assertion that the TL dating technique is inapplicable to the site of Caune de l'Arago due to instability in the 280 °C peak. For the oldest stalagmites from the site, it is possible that allowance may need to be made for signal fading; this would involve a small upward correction of the ages by $8 \pm 8\%$. Note, however, that more serious difficulties may be experienced when dating older stalagmites from warmer sites.

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Quantitative nannofossil correlation to open ocean deep-sea sections from Plio-Pleistocene boundary at Vrica, Italy

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Although numerous studies have been performed on material from the Vrica section, southern Italy^{1–3} with a view to establishing its suitability as a type section for the Plio-Pleistocene boundary, these studies have failed either to establish the age of the boundary, or to achieve accurate correlation to other regions. We report here quantitative studies of selected nannofossil species in this section which unambiguously correlate it with open ocean piston cores in which an accurate chronology has been established. The age of the Plio-Pleistocene boundary as proposed by Colalongo *et al.*⁴ is estimated to be ~ 1.6 Myr.

At the International Geological Congress held in London in 1948, a resolution was passed⁵ that the Plio-Pleistocene boundary should be placed at the base of the Calabrian Stage marine strata in Italy, at the first indication of cooling and the immigration of coldwater indicators such as *Hyalinea baltica*. At the 7th INQUA Congress in Boulder, Colorado in 1965 the section at Le Castella was discussed^{6,7} as a possible type section, and many studies have been made in that section^{8–12}. However, it became clear during these studies that neither the Le Castella section nor the nearby Santa Maria di Catanzaro section was ideal. In particular, a hiatus is reported in the region of the proposed boundary at Le Castella⁸.

At the 10th INQUA Congress in Birmingham in 1977, Selli *et al.*¹ reported on a new section at Vrica which appeared to be more suitable; being a deeper-water section it contains a better representation of the marine microfossils that should

permit accurate correlation to the open ocean and hence to other regions of the world. With respect to the previous boundary sections at Le Castella and Santa Maria di Catanzaro, Haq *et al.*⁹ estimated the age of the boundary to be ~ 1.6 Myr, equivalent to 1.66 Myr using the recently adopted¹³ decay constants for K–Ar. However, the criteria used for making this estimate were not quantified, so that although Selli *et al.*¹ regarded their newly proposed section as covering the same stratigraphical boundary as was studied by Haq *et al.*⁹, they considered that the age was close to 2.1 Myr on the basis of K–Ar and fission track measurements on ash samples in the Vrica section^{1,14}. Although Obradovich *et al.*¹⁵ have established that this age estimate is incorrect, the fact remains that biostratigraphical techniques as traditionally used were unable to resolve this problem unambiguously. Quantitative techniques have now been established¹⁵ which permit a definite conclusion to be reached.

Among the biostratigraphical datum levels that have been examined¹⁶, those relevant to the present problem are: the rise in abundance of the triradiate form of *Discoaster brouweri* (*D. brouweri* var. *triradiatus*) relative to the common variety; the simultaneous extinction of both these species, the extinction of *Calcidiscus macintyreii* and the extinction of *Helicosphaera sellii*. The extinction of *D. brouweri* has been shown to occur almost precisely at the lower boundary of the Olduvai normal magnetic subchron in all oceans and over a wide latitudinal range. Only through quantitative studies can it be established that the sporadic occurrences of this distinctive species at stratigraphically higher levels are the result of reworking. However, a second feature renders this boundary unusually reliable: a significant increase in the proportion of the triradiate variety occurs just before the extinction of both forms of the species. Although occasional examples of this form are found earlier in the range of *D. brouweri*, they are never abundant, so that there is not a reserve of the triradiate form of *D. brouweri* available for reworking. Takayama¹⁷ noticed anomalous numbers of the triradiate form of *D. brouweri* in the Le Castella section, but was unaware of their stratigraphical significance.

The extinction of *C. macintyreii* is also easily quantified. Studies in numerous cores have led us to conclude that this event occurred synchronously over a wide latitudinal range at ~ 1.45 Myr. The extinction of *H. sellii* is a less reliable datum since this species disappeared before *C. macintyreii* in mid-to-

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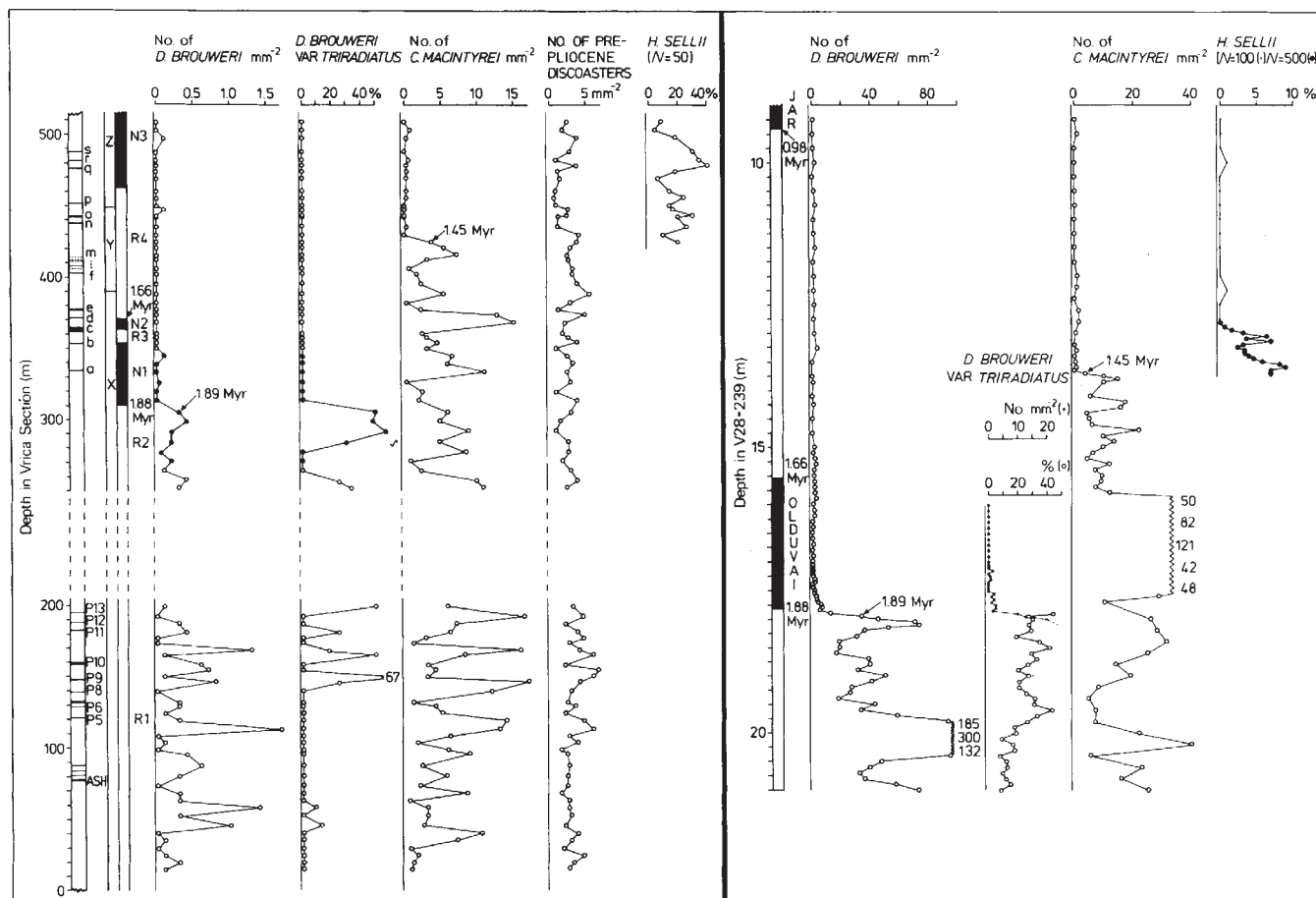


Fig. 1 Quantitative nannofossil stratigraphy of the Vrica section and of piston core V28-239. On the left is the lithological section at Vrica (from ref. 1), followed by the magnetostratigraphical record of Tauxe *et al.*²⁰. The nannofossil data shown are *D. brouweri*, *D. brouweri* var. *triradiatus* relative to all forms of *D. brouweri*, and *C. macintyreii*, for each of which a clear upper limit is observed; pre-Pliocene discoasters, which show a uniform working abundance through the section; and *H. sellii* as a proportion of all helicosphaerids, which does not show any drop in abundance in the upper part of the section. The filled circles between 270 and 345 m indicate samples that were prepared and counted in duplicate. To the right, the sequence in piston core V28-239 is shown (from ref. 16; magnetostratigraphy from ref. 18).

high latitudes, but later, at ~1.37 Myr in some low latitude cores¹⁵. The ages of these events were estimated by interpolation between magnetic reversals in deep-sea sediment cores, especially core V28-239¹⁸ in which the rate of sediment accumulation was very uniform; thus the ages depend ultimately on the K-Ar controlled time scale for magnetic reversals¹³.

We have not studied the evolution of *Gephyrocapsa* spp.¹⁶ because we consider that the detailed study by Samtleben¹⁹ shows that this group cannot be used without a considerable amount of further work. In any case our studies suggest that extinction data, when defined in a consistent manner, represent a more accurate means of correlation than evolutionary appearance datums.

Figure 1 shows our data for the Vrica section, together with the data from piston core V28-239^{16,18}, one of the best-known of the many cores in which we have performed these studies. Selli *et al.*¹ proposed that the Plio-Pleistocene boundary should be placed within the unit designated Y, while Colalongo *et al.*⁴ proposed more recently that the base of this unit, marked by the first appearance of *Cytheropteron testudo*, should be taken as the boundary. Our studies suggest:

- (1) The age boundaries of Unit Y are ~1.4 and 1.6 Myr, estimated on the basis of a uniform accumulation between the clearly defined extinction levels of *D. brouweri* (1.89 Myr) and of *C. macintyreii* (1.45 Myr).
- (2) The magnetozones N1, N2 identified in the study of Tauxe *et al.*¹⁹ correspond to the Olduvai magnetically normal subchron.
- (3) The ages of the boundaries of this normally magnetized sediment implied by our biostratigraphical data are consistent

with the ages for the boundaries of the Olduvai Subchron¹³ on which these biostratigraphical data were calibrated, so that there is no evidence for either reversal boundary in this section representing a hiatus.

(4) *H. sellii* survives *C. macintyreii* in this section, so that it is unlikely that the extinction level of *C. macintyreii* represents a hiatus.

(5) *H. sellii* apparently survives into magnetozones N3. Although the whole section is marked by very extensive reworked influx of Palaeogene and Cretaceous nannofossils, the possibility of the *H. sellii* specimens being reworked must be seen in the context of reworking levels for sediments in the time range of this species, and are best judged by the reworked *C. macintyreii* and *D. brouweri* specimens; in this light, reworking is an unlikely cause for the presence of these specimens. Thus magnetozones 3 may not represent the Jaramillo subchron but may instead represent sediment magnetized during a short normal interval earlier in the Matuyama. This indication has to be regarded as tentative in view of our reservations on the reliability of the extinction of *H. sellii* as a datum. It appears that the accumulation rate must be higher in the upper part of the section in view of the long upward extension of *H. sellii* specimens.

(6) In view of our conclusion that there is no evidence that any of the biostratigraphical or magnetostratigraphical marker horizons in the immediate neighbourhood of the proposed boundary lies at a hiatus, Unit Y of the Vrica section is suitable for use as a type section for the Plio-Pleistocene and Neogene-Quaternary boundary.

(7) The most accurate biostratigraphical marker available for global correlation of this event in marine sediments is the

extinction of *C. macintyre*: we recommend that this datum be adopted as the most convenient biostratigraphical approximation to the Plio-Pleistocene boundary in marine sections. The age of this datum is 1.45 ± 0.05 Myr (ref. 16). However, we estimate that the age of the proposed Neogene-Quaternary boundary at the base of layer Y with the first *C. testudo* as ~ 1.6 Myr, and the age of the top of layer Y marked by the first *Hyalinea baltica* as ~ 1.4 Myr.

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'Unlearning' has a stabilizing effect in collective memories

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Crick and Mitchison¹ have presented a hypothesis for the functional role of dream sleep involving an 'unlearning' process. We have independently carried out mathematical and computer modelling of learning and 'unlearning' in a collective neural network of 30-1,000 neurones. The model network has a content-addressable memory or 'associative memory' which allows it to learn and store many memories. A particular memory can be evoked in its entirety when the network is stimulated by any adequate-sized subpart of the information of that memory². But different memories of the same size are not equally easy to recall. Also, when memories are learned, spurious memories are also created and can also be evoked. Applying an 'unlearning' process, similar to the learning processes but with a reversed sign and starting from a noise input, enhances the performance of the network in accessing real memories and in minimizing spurious ones. Although our model was not motivated by higher nervous function, our system displays behaviours which are strikingly parallel to those needed for the hypothesized role of 'unlearning' in rapid eye movement (REM) sleep.

In the most symmetric form of collective memory in our dynamic neural network², each neurone, j , has two states, and is described by a variable $\mu_j = \pm 1$. The instantaneous state of the system of N neurones can be thought of as an N -dimensional vector having components μ_i of size 1. The neurones are interconnected by a network of synapses, with a synaptic strength T_{ij} from neurone j to neurone i . The instantaneous input to neurone i is

$$\text{input to } i = \sum_{j \neq i} T_{ij} \mu_j$$

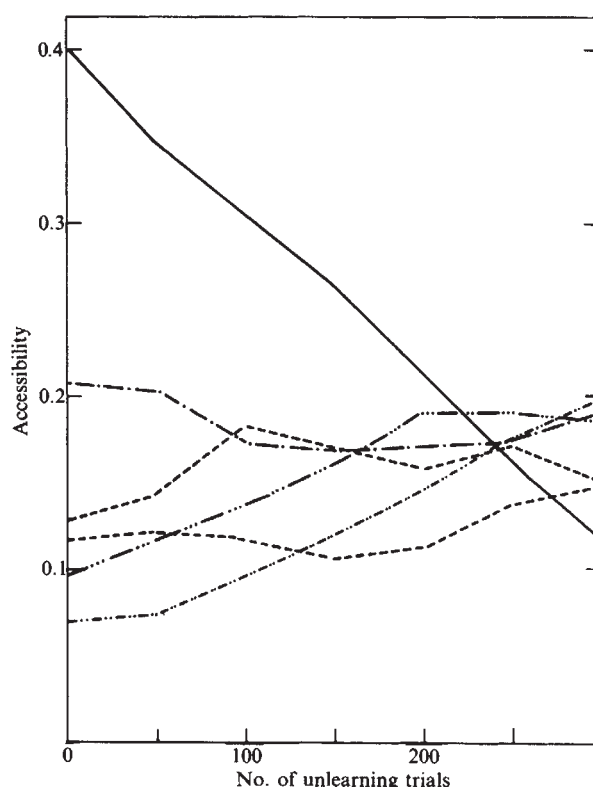


Fig. 1 The fraction of random starting states which leads to particle memories (accessibility). The five dashed lines are the five nominal memories. The solid line is the total accessibility of all spurious memories. In these trials ϵ was set at 0.01.

where μ_j is the present state (± 1) of neurone j . The neural state of the system changes in time under the following algorithm. Each neurone i interrogates itself at random in time, but at a mean rate W , and readjusts its state, setting $\mu_i = \pm 1$ according to whether the input to i at that moment is greater or less than zero. The neurones act asynchronously.

This algorithm defines the time evolution of the state of the system. For any symmetric connection matrix, there are stable states of the network of neurones, in which each neurone is either 'on' and has an input ≥ 0 or 'off' and has an input < 0 . These stable states will not change in time. Starting from any arbitrary initial state, the system reaches a stable state and ceases to evolve in a time of $\sim 3/W$.

The stable states of the system can be arbitrarily assigned by an appropriate choice of T_{ij} . Suppose n different N -dimensional state vectors

$$\mu_i^s \}_{i=1}^N \quad s = 1 \text{ to } n \leq 0.25N$$

are to be stable states of the system. If these state vectors are sufficiently different, and if the synaptic connection matrix T_{ij} is given by

$$T_{ij} = \sum_s \mu_i^s \mu_j^s; \quad T_{ii} = 0 \\ i \neq j$$

then the states μ^s will be stable states of the system.

This network now functions as an associative memory. If started from an initial state which resembles somewhat state μ^t and which resembles other μ^s ($s \neq t$) very little, the state will evolve to the state μ^t . The states μ^s are evokable memories, and the system correctly reconstructs an entire memory from any initial partial information, as long as the partial information was sufficient to identify a single memory. Detailed properties of the collective operation of this network have been described previously².

The form of the T_{ij} matrix can be described as an incremental learning rule. To learn a new memory μ^{new} , increment T_{ij} by

$$\text{learn } \mu^{\text{new}} \Delta T_{ij} = \mu_i^{\text{new}} \mu_j^{\text{new}}$$

In biology or in circuits, this would be done by placing the system in state μ^{new} —for example, driven by external inputs—and enabling a learning process that allows all T_{ij} to increment. The information needed by each synapse is local—the increment for synapse ij does not depend on the global structure of the new state or past memories, but only on μ_i^{new} and μ_j^{new} .

Under this algorithm, when random starting states are chosen, some stored memories are much more accessible than others, that is, considerably larger numbers of randomly chosen initial states lead to some memories than to others. This is a vagary of the particular set of memories which have been learned. It occurred to us that it would be possible to reduce this unevenness of access (which can be intuitively described as the "50% of all stimuli remind me of sex" problem) by 'unlearning'.

Specific unlearning was implemented by choosing starting states at random; when a final equilibrium state μ^f was reached it was weakly unlearned by the incremental change

$$\text{unlearn } \mu^f \Delta T_{ij} = -\varepsilon \mu_j^f \mu_i^f, 0 < \varepsilon \ll 1$$

Figure 1 illustrates the effect of unlearning on the accessibility of five stored memories in a set of 32 neurones. Accessibility is quantitatively defined as the fraction of random initial states leading to a particular final stable state or group of states. The unevenness of the lines is due in part to statistical noise in the simulation. The accessibility of the nominal assigned memories initially ranges over a factor of 3, but converges with unlearning to a spread of only a factor of 1.4. Thus the accessibility is much more uniform (or in Crick-Mitchison terms, the relative stability of the modes made more uniform) after specific unlearning, and the system will have functionally improved recall.

In our model the storage of a set of assigned memories in T_{ij} also produces a set of spurious stable states which were not inserted as memory states. One of the strong effects of unremem-bering is to reduce the total accessibility of spurious states, as shown by the solid line in Fig. 1.

The qualitative reason for the success of unlearning comes from the behaviour of the 'energy' E , defined for any state μ as

$$E = - \sum_{i \neq j} \sum_i T_{ij} \mu_i \mu_j$$

The change of neural state with time according to the asynchronous algorithm monotonically decreases E until a final stable state is reached—either a stored memory or a spurious memory. Any stable state μ^m has, for a given T_{ij} , an energy E^m . There is a strong tendency for the states having the deepest energy valleys to collect from the largest number of random starting states, that is, deep valleys are also broad. When a final state μ^f is unlearned, its energy E^f is specifically raised and its valley of collection diminished relative to other states. While this argument indicates why accessibility of stored memories should be made more nearly even by unlearning, only a detailed analysis shows why the spurious states should be so sensitive to it. Too much unlearning will ultimately destroy the stored memories.

We have identified a class of spurious states, which in their most elementary form have their origin in triples. As an example on 16 neurones

Memory 1	+	+	+	+	-	-	-		+	+	-	+	-	+	-
Memory 2	+	+	+	+	-	-	-		-	-	+	-	+	-	+
Memory 3	+	+	+	-	-	+	+		+	+	-	+	+	-	+
Spurious memory	+	+	+	+	-	-	-		+	-	-	+	+	-	+

The stability of the spurious memory is enhanced if the first half of memory 3 is weakly correlated with memories 1 and 2. Mathematical analysis of the statistical stability of such spurious states shows that they are typically less stable than the assigned memories, and that the stability will also depend on correlations with other memories. The nature of these spurious states can be described by analogy in terms of higher level function by

the example

Memory 1	Walter, white
Memory 2	Walter, black
Memory 3	Harold, grey
Spurious memory	Walter, grey

where grey is taken as a category equally resembling black and white. This spurious state is more stable when 'Harold' and 'Walter' have a significant correlation—perhaps 'Harold' and 'Harry'. These particular spurious states are not simply transitive logical associations of the form $A \leftrightarrow B$, $B \leftrightarrow C$; $\rightarrow A \leftrightarrow C$. They are truly spurious 'illogical' associations, but perhaps 'plausible' as they come from correlations in the structure of memories.

In our simple system, unlearning improves memory function both by the equalization of accessibility and the suppression of spurious memories. We asked whether other simple algorithmic changes such as clipping the T_{ij} matrix or a threshold effect produce an equivalent improvement in memory performance. These two do not, presumably because they lack the essential element of the present scheme, that is, the feedback via the algorithm of information about the accessibility of particular states. We believe the results found will be insensitive to whether the state component values are taken as 0 and 1 or ± 1 .

The REM sleep hypothesis of Crick and Mitchison¹ refers to higher level processing. Our example illustrates that from a mathematical viewpoint the general idea could work as they described. If the Crick-Mitchison hypothesis is correct, one might ask about correlations between the structure of the spurious linkages in modelling and the strange associations present in dreams.

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A language-specific comprehension strategy

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Infants acquire whatever language is spoken in the environment into which they are born. The mental capability of the newborn child is not biased in any way towards the acquisition of one human language rather than another. Because psychologists who attempt to model the process of language comprehension are interested in the structure of the human mind, rather than in the properties of individual languages, strategies which they incorporate in their models are presumed to be universal, not language-specific. In other words, strategies of comprehension are presumed to be characteristic of the human language processing system, rather than, say, the French, English, or Igbo language processing systems. We report here, however, on a comprehension strategy which appears to be used by native speakers of French but not by native speakers of English.

Underlying our finding is a structural difference between the two languages; French and English differ considerably in the degree to which syllable boundaries are clear and unambiguous. In French, syllabic structure is relatively easily determined¹;

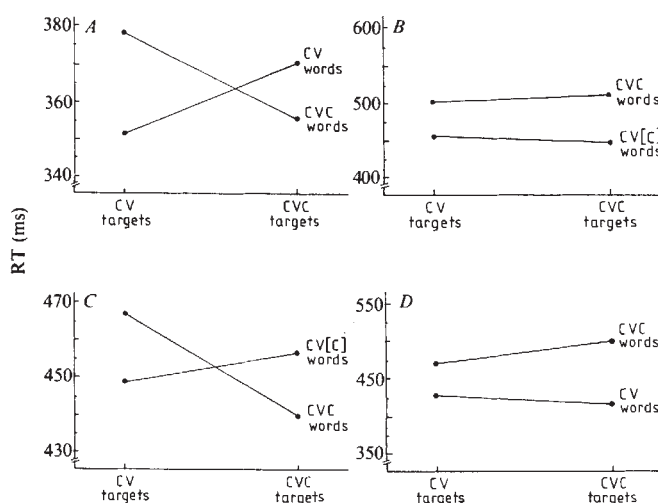


Fig. 1 Mean target detection response time (RT) as a function of size of target sequence (CV, for example, *ba-* versus CVC, for example, *bal-*) and size of initial syllable of stimulus word (CV versus CVC for French; CV[C] versus CVC for English), for the following permutations of subjects' native language and stimulus presentation language. A, French subjects and words; B, English subjects and words; C, French subjects, English words; D, English subjects, French words.

the first syllable of *balance*, for example, is clearly *ba*, the first syllable of *balcon* clearly *bal*. In English, however, syllable boundaries are often unclear²; although English speakers agree that *balcony* has a syllable boundary after *bal*, the syllable boundary in *balance* falls neither after *ba* nor after *bal*. Anderson and Jones² would represent the first syllable as [*ba*], the second as [*lance*], the whole word as [*ba*lance]; the 'l' properly belongs to both the first and the second syllable. Segments which belong to two syllables at once are said to be ambisyllabic; below, we shall use [C] to represent an ambisyllabic consonant.

Previous work³, using French words, indicated that in language perception the syllable functions as an effective processing unit; incoming words are processed syllable by syllable. Mehler *et al.*³ asked University of Paris students to listen to lists of unrelated words and to press a response key as fast as possible when they heard a specified word-initial sequence of sounds. This target was either a consonant-vowel (CV) sequence such as *ba-* or a consonant-vowel-consonant (CVC) sequence such as *bal-*. The words which began with the sequence had one of two syllabic structures: the initial syllable was either open (CV), as in *balance*, or closed by a consonant (CVC), as in *balcon*. They found that response time was significantly faster when the target sequence corresponded exactly to the initial syllable of the word than when the target sequence was equal to more or less of the word than the initial syllable. Thus, the response to the target *ba-* was faster in the word *balance* than in *balcon*, while the target *bal-* was responded to faster in *balcon* than in *balance* (see Fig. 1A).

This syllabification effect has also been demonstrated in many other studies by Mehler, Segui and colleagues, using a variety of experimental techniques^{4,5}. Moreover, sensitivity to the syllable as a unit seems to be natural for pre-linguistic infants⁶, which suggests that it may be a characteristic of the human language processor, independent of particular languages.

Perceptual strategies such as syllabification have presumably been developed because they can speed up the recognition of words. We do not know, however, whether this particular strategy would be as efficient for English as it appears to be for French. Because ambisyllabicity is prevalent in English, and syllable boundaries are therefore hard to identify, it is likely that syllabification would frequently prove difficult, so that it would tend to make the comprehension process less rather than more efficient. Therefore, we replicated the study of Mehler *et al.*³ using English material, and English-speaking subjects.

Twenty-four students at the University of Sussex listened for either CV or else CVC targets in lists of isolated words; the target-bearing words' initial syllables had either CV[C] (for example, *balance*) or CVC (for example, *balcony*) structure. The results are presented in Fig. 1B; clearly, the response time to CV and CVC targets was not significantly different either in CV[C] or in CVC words.

Thus, the structural difference between French and English seems to be reflected in a difference in the way in which the two languages are processed. The syllabification strategy works efficiently in the perception of French, because French is easy to divide into syllables; hence, the syllabification strategy is used by French listeners. English, however, is hard to syllabify, so that such a strategy would be highly inefficient in perception; and indeed, English listeners do not use it. Our results appear to show, therefore, that human listeners use different processing strategies with different languages.

The question then arises of whether such strategies are characteristic of the perceiver (that is, will syllabification be used by French listeners but not by English listeners irrespective of input language?), or whether they are imposed by the characteristics of the speech material (that is, will syllabification be used in the perception of French but not of English irrespective of the perceiver's native language?).

Therefore, we conducted two further experiments in which the subjects' native language and stimulus presentation language differed. Twenty-four University of Sussex students, with only rudimentary knowledge of French, performed the sound sequence detection task on the French material used in the study of Mehler *et al.*³. The results (see Fig. 1D) showed that the performance of the English listeners hearing French material was strikingly similar to that of English listeners who had listened to English material in the previous study. Similarly, when the material from the English-language study was presented to 20 University of Paris V students who were not fluent in English, the performance of these subjects was strikingly similar to the performance of the French listeners who had heard French material in the Mehler *et al.* study (see Fig. 1C), despite the fact that the language material did not in this case lend itself to the syllabification strategy.

We conclude, therefore, that the syllabification strategy is characteristic of listeners rather than of stimulus language. We suggest that listeners who have acquired French as their native language have developed the syllabification procedure, natural to the human language processing system, into an efficient comprehension strategy. On the other hand, listeners whose native language is English, where this strategy would not necessarily achieve greater comprehension efficiency, have not included syllabification in their repertoire of processing strategies. Further, we suggest that syllabification is only one of a range of possible strategies which the newborn brain has the potential to acquire; whether or not a particular strategy is incorporated in the developing language user's comprehension system will depend on the degree to which the structure of the language being acquired encourages the use of the strategy in question.

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Topographical rearrangement of acetylcholine receptors alters channel kinetics

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Plasma membranes are dynamic structures of proteins and lipids. Protein-protein or protein-lipid interactions within the membrane are believed to have important roles in many membrane functions, including ion transport, enzyme activity and signal reception^{1,2}. The acetylcholine (ACh) receptor-channel complex in skeletal muscle membrane is one of the best known integral membrane proteins³. Its ion transport function is accessible to direct measurement at the single-channel level by the use of the 'giga-seal' patch recording technique⁴. Here we used an *in situ* electrophoresis technique⁵ to rearrange the topography of pre-existing ACh receptor-channels in the muscle membrane, and measured the single-channel kinetics of ACh-activated channels in two different molecular environments within the membrane: those in the diffusely distributed region and those in the ACh receptor clusters induced by the applied field. We found that the channel kinetics are significantly prolonged in the ACh receptor cluster compared with the non-clustered region of the same cell. This result strongly supports the notion that the function of a membrane ionic channel depends on the local molecular environment.

Experiments were carried out on dissociated embryonic *Xenopus* myotomal muscle cells in culture⁶. Application of a uniform electric field of 2–3 V cm⁻¹ to a 12-h-old culture for 12–14 h consistently produced large ACh receptor (AChR) clusters on the cathode-facing pole of the spherical muscle cells (Fig. 1), as shown by post-field specific labelling of cell-surface AChR with fluorescently conjugated α -bungarotoxin (α -BGT). These AChR clusters persisted for at least 5 h after the field had been removed, indicating that the field-induced AChR clusters are relatively stable. Application of the field in the presence of concanavalin A (Con A), a lectin known to block lateral mobility of AChRs in these muscle cells^{7,8}, abolished the formation of clusters. Application of a 10 V cm⁻¹ field for 3 h on a 1-day-old culture produced similar stable clustering of AChRs, even in the continuous presence of metabolic inhibitors (1 mM dinitrophenol, 10 mM sodium azide and 10 mM sodium fluoride). Taken together, these results indicate that the electric field caused formation of large, stable AChR clusters by lateral rearrangement of pre-existing AChRs on the muscle surface.

Kinetics of single AChR channels were measured with a 'giga-seal' patch electrode containing a low concentration of ACh. For comparison of AChR channel kinetics in the clustered and non-clustered states, recordings of single-channel current through muscle membrane were made at the cathode-facing and anode-facing poles of the field-treated spherical muscle cells. Consistent with results from the α -BGT labelling studies, the channel density on the cathode-facing pole was generally higher than that of the anode-facing pole, as indicated by higher channel opening rates and more multiple channel events. Figure 2a,b shows double exponential fits to the distribution of channel burst durations from data recorded on the cathode- and anode-facing poles of the same cell in a field-treated culture. The mean channel burst duration was found to be significantly lengthened at the cathodal pole. Figure 3 depicts results from nine pairs of recordings from cathode- and anode-facing poles of cells from nine different cultures. Seven of the nine pairs showed significant lengthening of mean channel burst duration on the cathode-facing pole compared with the anode-facing pole. The average ratio of mean burst durations on the two

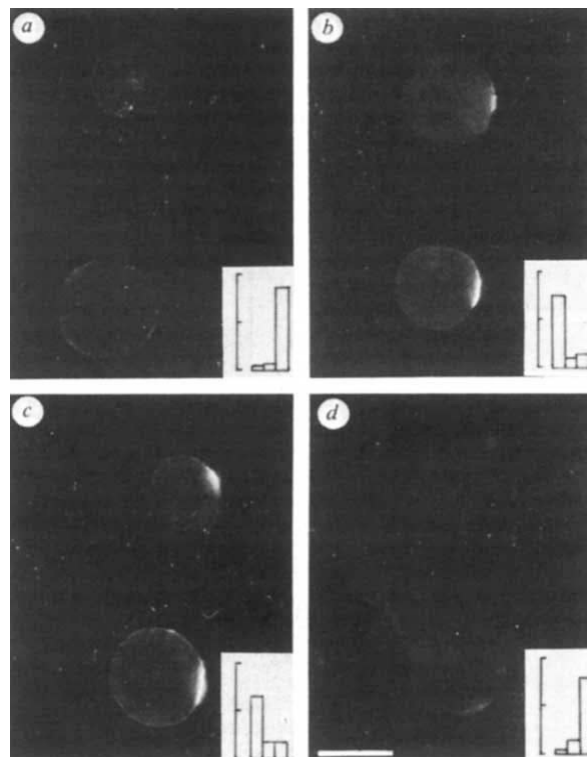


Fig. 1 Clustering of acetylcholine receptors (AChRs) on the surface of cultured *Xenopus* muscle cells induced by an electric field. The AChRs were visualized by staining the cells with α -bungarotoxin conjugated with tetramethylrhodamine. Insets: cell scoring for cluster formation. Left bar, percentage of cells showing large AChR clusters (size $\geq 5 \mu\text{m}$) within a 30° divergence angle of the cathode-facing pole; centre bar, percentage of cells with large clusters outside the 30° angle; right bar, percentage of cells showing absence of large clusters. Scale on ordinate: 0, 50 and 100%. *a*, Representative cells in 1-day-old control culture show uniform diffuse staining as well as numerous small 'microclusters'. Cell scoring for 208 cells: 5, 7, and 88%. *b*, Cells from a 1-day-old culture that had been exposed to a 2 V cm⁻¹ field for 12 h at 22 °C before fluorescence labelling. Cathode of the field is located to the right of this and subsequent figures. Large clusters of AChR appeared on the cathodal poles and there were few microclusters. Cell scoring for 126 cells: 76, 10 and 14%. *c*, Cells treated identically with those in *b* except that the labelling was delayed for 5 h after termination of the field. This demonstrates the stability of AChR clusters in the absence of the field. Cell scoring for 94 cells: 67, 17 and 16%. The decrease in number of clusters oriented towards the cathode was probably due to cell rotation during the 5-h relaxation period, as the total fraction of cells containing large clusters was similar in *b* and *c* (86% versus 84%). *d*, Cells treated identically to those in *b* except 10 $\mu\text{g ml}^{-1}$ Con A was added to the incubating medium during application of the field. The presence of Con A prevented the clustering of AChRs. Note also the lower staining intensity and disappearance of microclusters. Cell scoring for 136 cells: 5, 15 and 80%. Scale bar, 20 μm .

Methods: Preparation of the embryonic muscle cells from embryos of *Xenopus laevis* and method of *in situ* electrophoresis were identical to those previously reported^{6,7}. We used a 1-day-old culture because previous experiments have shown that the slow to fast conversion of AChR channel kinetics normally occurs at a later stage^{10,13}. Fluorescence labelling of AChRs was done by incubating the cells with Steinberg's saline containing fluorescent α -BGT for 1.5 h. This was followed by extensive rinsing with saline containing 1% bovine serum albumin. Cells were then fixed in cold acetone and preserved in 95% glycerol before observation and photography on a Zeiss fluorescence microscope.

poles for these seven cases was 1.69 ± 0.09 (s.e.). The two cases which did not show any lengthening effect may be accounted for by the fact that 24% of the cells in the culture fail to exhibit large AChR clusters on the cathode-facing pole after the same field treatment (see Fig. 1*b*). The mean burst durations at the anodal poles do not differ significantly from control values, an

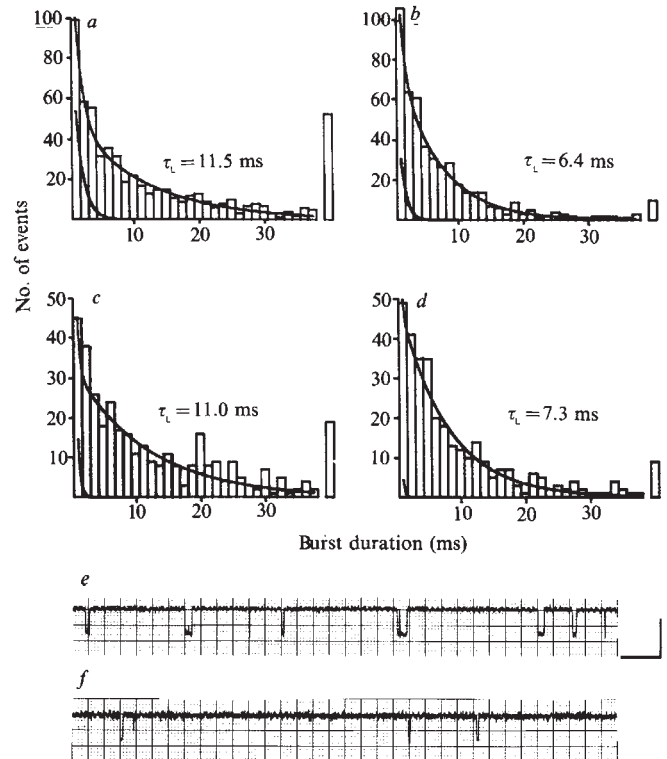
Fig. 2 Mean burst duration of AChR channels was prolonged on the cathodal pole of field-treated muscle cells. Distributions of channel burst times are plotted in histograms. The solid lines are weighted, least-squares fits of the sum of two exponentials to the envelopes of the histograms. The short time constant component alone is shown in the bottom corner of each histogram. The long time constant, τ_L , yields the mean burst duration. Single bars at the right edge of each histogram represent the number of single-channel events having burst durations >25 ms. *a, b*, Data from the cathodal (*a*) and anodal (*b*) pole of the same cell in a 1-day-old culture that had been exposed to a 2 V cm^{-1} field for 12 h before recording. The ratio of mean burst durations is 1.79. *c, d*, Data from cathodal (*c*) and anodal (*d*) poles of two cells in the same culture treated with a mixture of metabolic inhibitors (1 mM dinitrophenol, 10 mM sodium azide, 10 mM sodium fluoride) 1 h before and during application of a 10 V cm^{-1} field (3 h). The shorter exposure time (and higher field strength) was necessary due to decreased viability of the cells with 12 h exposure to metabolic inhibitors. Lengthening of channel kinetics was observed. The ratio of mean burst duration on the two opposite poles was 1.51. *e, f*, Representative recordings of single-channel currents from cathodal (*e*) and anodal (*f*) poles of two cells that had been treated identically to that in *c* and *d*. Scale bars, 100 ms, 2.0 pA.

Methods: Recording. Single-channel current recordings were made using the 'giga-seal' technique⁴. The recording pipette contained (in mM) NaCl 116, KCl 0.7, $\text{Ca}(\text{NO}_3)_2$ 0.4, MgSO_4 1.3, HEPES 5, pH 7.8, and 33–100 nM of acetylcholine chloride. The bath solution had the same composition but without acetylcholine chloride. All recordings were performed at 10°C . Although only one recording can be made by each pipette, an attempt was always made to record channel kinetics on both the cathode-facing and anode-facing poles of the same cell or cells in the same culture, using pipettes of similar resistance (2–5 M Ω) and filled with the same concentration of ACh. **Analysis.** High resolution recording has shown that channel openings can be interrupted by brief closures, producing a burst of openings¹⁵. The time course of the bursts rather than the individual openings was used in the analysis because it is the mean burst duration which determines the relaxation of membrane current and which produces the corner frequency obtained from current noise analysis^{15–17}. A burst was defined as a series of openings separated by closed times of less than 1 ms. The burst duration histograms were fitted by the sum of two exponentials, but we are interested only in the long time constant component, which is the major determinant of mean burst duration^{16,17}. **Channel subtypes.** Our analysis of mean burst duration was based on the assumption of a single AChR channel type. We have observed, as have others^{18–20}, that there exist two classes of AChR channels of different conductances. The existence of a bimodal distribution of conductance states may affect interpretation of our data in that two-component exponential decays might not be the best fit to our burst time distributions; and the change in kinetics observed in the AChR clusters might be due to an electrophoretic separation of channel types. We found that in control cells (no field), the ratio of the average amplitudes of the two channel types was 1.32, with the small conductance channel being more abundant (81%). Channels recorded at the cathodal pole of the field-treated cells showed similar subdivision of channel types, with a conductance ratio of 1.35, and smaller channels were more abundant (75%). The similarity in the pattern of subdivision of channel types in control cells and in field-induced clusters suggests no preferential segregation of the channel types had occurred; and treating the two channel types separately seems unnecessary.

indication that the field itself has no direct effect on channel kinetics. Combining all experiments using 12-h field treatment, we have recorded from AChR channels on 13 cathode-facing poles and 10 anode-facing poles, including the nine pairs of recordings shown in Fig. 3. 12 parallel control cells (no field treatment) were also studied. The ratios of the grand average mean burst durations (cathode/anode/control) were 1.61:1.11:1.00.

As an independent test of our findings, we simply counted the percentage of channel events with burst durations longer than 25 ms. The result of this analysis clearly indicates that significantly more longer-duration events were observed in the cathode-facing pole of the cells ($14 \pm 2\%$, $n = 13$) than in the anode-facing pole of the cells ($6 \pm 1\%$, $n = 10$) or the control cells ($5 \pm 1\%$, $n = 12$).

The lengthening of channel mean burst duration at the cathodal pole cannot be attributed to variation in cell membrane potential or bath temperature during the recording (see Fig. 3 legend), but correlates with the clustering of AChRs. Incubation of the culture with $10 \mu\text{g ml}^{-1}$ Con A during the application of a 12-h field, a treatment that blocked clustering of AChRs by the field (Fig. 1*d*), abolished the lengthening effect; the average ratio of mean burst durations recorded at cathodal and anodal poles was 0.95 ± 0.05 ($\pm$ s.e., $n = 4$). Treatment with Con A reduced the AChR channel opening rate about 20-fold, while the mean burst duration was unaffected: the average ratio of mean burst duration for Con A-treated to non-treated cells was 0.96.



The lengthening of channel burst duration in the field-induced clusters was not due to a preferential incorporation of a new channel type into the cathodal side of the membrane, but rather resulted from the lateral rearrangement of pre-existing channels in the membrane. In two separate experiments, all the pre-existing AChRs on the cell surface were first blocked with α -BGT ($50 \mu\text{g ml}^{-1}$, 30 min incubation). The cultures were then extensively washed with fresh saline and exposed to a field of 2 V cm^{-1} for 12 h. Post-field single-channel measurements showed that the average channel opening rates remained less than 1% of those cells not pretreated with the toxin (three cells in two separate cultures), indicating that very few new AChR channels were incorporated into the membrane during the 12-h period. This slow rate of channel incorporation is consistent with the reported long turnover half time (50 h) of AChRs in these *Xenopus* muscle cells⁹. Finally, we found that the lengthening of burst kinetics also occurred in cells under prolonged metabolic inhibition, a condition that may block energy-dependent incorporation of new channels. In two out of three pairs of recordings, channel kinetics were found to be significantly prolonged on the cathode-facing poles (see Fig. 2*c, d*). The ratios of mean burst duration on the two opposite poles of the cells were 2.04, 1.51 and 0.84.

During maturation of the neuromuscular synapse, AChRs diffusely distributed over the surface of the muscle become clustered at the postsynaptic membrane. In rat and frog muscle, this change in AChR distribution is accompanied by a change in the population of ACh-activated channels from one of

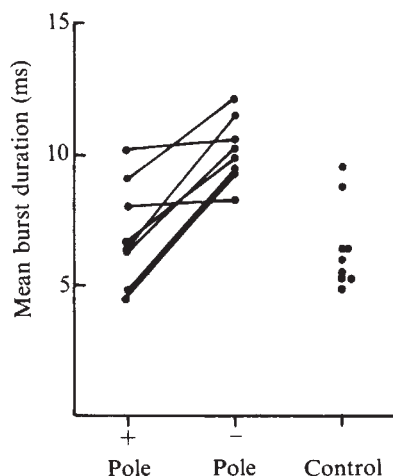


Fig. 3 Comparison of AChR channel mean burst durations in clustered and non-clustered states. Nine pairs of recordings were made in nine different 1-day-old cultures that had been exposed to an electric field of 2 V cm^{-1} for 12 h. Each pair consists of recordings at the anodal and cathodal poles of one cell or two different cells in the same culture. Mean burst durations were also obtained from nine parallel (no field) control cultures. Solid lines join data from paired recordings. Seven out of nine pairs showed significant lengthening of burst duration at the cathodal pole. Only one pair of recordings was made on each culture, and the sequence of recording (cathode first or anode first) was varied. The mean amplitude of single-channel current varied from culture to culture (1.6–2.2 pA), but no data were used in which anodal and cathodal amplitudes differed by more than 20% within the same pair of recordings. Temperature variation during the experiments, as measured by a thermistor in the bath, was $\pm 0.5^\circ\text{C}$, too small to affect channel kinetics significantly.

primarily 'slow' channels to one of primarily 'fast' channels^{10,11}. Sakmann and co-workers have provided evidence that the conversion of slow to fast channel kinetics in the rat neuromuscular synapse is not due to incorporation of new channel types^{11,12}, but is more probably the result of some modification of the pre-existing channels or of their immediate environment. In the present study, field-induced clustering of pre-existing AChRs was shown to change their channel kinetics, but in a direction opposite to that which occurs during AChR clustering *in vivo*. Brehm *et al.*¹³ have recently found that the addition of nerve to the *Xenopus* muscle culture, which also induces clustering of AChR, in these muscle cells¹⁴, impeded the conversion of slow channels to fast channels. Taken together, it now seems that molecular events other than a simple receptor clustering must be involved in the alteration of channel kinetics at the synapses. AChR clusters observed in culture, whether induced by nerve or by other artificial means, may possess rather different molecular properties from those of the AChR clusters at the mature neuromuscular junction *in vivo*.

In conclusion, we have shown that the behaviour of an ionic channel can depend on its topographical arrangement in the plasma membrane. Specific molecular interactions with other membrane components are likely to affect its kinetics, and perhaps such specific interaction is responsible for the change of AChR kinetics during synapse maturation *in vivo*.

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Sleep deficits in rats with hereditary diabetes insipidus

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Interest in the Brattleboro diabetes insipidus rat has resurged with the recent increase in research on brain peptides. Various reports have suggested that in these rats, the impaired ability for memory consolidation is due essentially to a chronic lack of vasopressin¹. On the other hand, sleep stages and particularly the paradoxical phase of sleep have been shown to have a key role in the processes of learning and memory consolidation. Curiously, the possible involvement of sleep deficits in the impairment of memory function in the Brattleboro rat has never been suspected. Here I report a significant reduction (38%) in the daily duration of paradoxical sleep (PS) in the homozygous diabetes insipidus (HODI) rat compared to the heterozygous Long Evans strain. Normal or increased durations of PS were observed after intravenous (i.v.) administration of vasopressin but also when the normal daily water intake (240 ml) was infused i.v. These results provide direct evidence that PS deficits in the HODI rat are not due to the absence of vasopressin. They also suggest that the impaired ability for learning and memory processes are probably due to the impairment of PS rather than to some direct consequence of the hereditary lack of vasopressin.

Vasopressin, together with oxytocin, has been implicated in brain processes related to memory function in normal rats by several authors using different experimental paradigms^{2,3}. On the other hand, several authors have shown that PS is involved in learning and memory processes; ample evidence for this assertion has been assembled by using passive avoidance techniques⁴, active avoidance paradigms⁵ or conditioned taste aversion learning⁶. To distinguish between these possibilities, we have studied sleep patterns in HODI rats to investigate whether their impaired memory functions are due to vasopressin or to some sleep alterations.

Male HODI and Long Evans (INSERM U90, Hôpital Necker, Paris) rats weighing 200–300 g, were implanted with chronic cortical electrodes for electrocorticographic (ECG) sleep recordings. During the same surgical session for the HODI rats, a permanent cannula was also implanted in the jugular vein for continuous i.v. infusions. This cannula was then cemented to the skull adjacent to the ECG microconnector. When food and water intakes and body weight had returned to preoperative levels the rats were transferred to Plexiglas cages and maintained on a 12 h/12 h lighting cycle (08.00–20.00 h) in a temperature-regulated ($24 \pm 1^\circ\text{C}$) room with food and water available *ad libitum*. In normal conditions the daily duration of PS was significantly lower (38%) in HODI rats compared to that in Long Evans rats (Fig. 1). However, there was no significant difference in the daily duration of slow wave sleep (SWS) between the two groups of rats (Fig. 1). As far as the circadian distribution of sleep is concerned, the results

Table 1 Circadian distribution of sleep in Long Evans and HODI rats

		LE (5)		HODI (10)	
		C	C	Vasopressin i.v.	Water i.v.
SWS (min)	Day	354 ± 12	296 ± 10*	376 ± 14‡	367 ± 22‡
	Night	229 ± 8	261 ± 19	321 ± 32‡	316 ± 7‡
PS (min)	Day	73 ± 4	43 ± 4†	70 ± 8§	73 ± 6§
	Night	36 ± 3	33 ± 4	55 ± 5§	53 ± 6§

Total durations of slow wave sleep (SWS) and paradoxical sleep (PS) expressed as mean $\pm$ s.e.m. for Long Evans (LE) rats in control (C) conditions and for HODI rats in control (C) conditions, following continuous i.v. infusion of vasopressin, or when the daily water intake was infused i.v. during the day (08.00–20.00) and night (20.00–08.00) phases of the circadian cycle. Compared to LE rats, both SWS and PS were significantly reduced essentially during the day in HODI rats thus resulting in an almost equal circadian distribution of sleep. Note that drinking episodes also were almost equally distributed between day and night in HODI rats. The continuous i.v. infusions of either water or vasopressin in HODI rats resulted in a significant increase of SWS and PS during the day as well as during the night. Numbers in parentheses are numbers of animals.

* $P < 0.05$; † $P < 0.01$ (Student's *t*-test, Long Evans versus HODI data in control conditions).

‡ $P < 0.05$; § $P < 0.01$ (paired *t*-test, control versus experimental data of HODI rats).

showed that, contrary to data obtained from Long Evans rats, both SWS and PS were almost equally distributed throughout the nycthemeron (Table 1). The reduction in the duration of PS could be directly related to the absence of vasopressin. Another explanation seems plausible, however, because the HODI rats spend a disproportionately large amount of their time drinking, their sleep is therefore often interrupted thus resulting in shorter SWS episodes and a decreased number of PS episodes. To test these possibilities, two experimental paradigms were used. First, a group of five HODI rats received a continuous i.v. infusion of vasopressin (0.48 μ g of a 4 μ g per 100 ml solution per day) while sleep was recorded on three consecutive days. The simultaneous ECG recordings and i.v. infusions were made possible by using a multiple swivel system (electrical for ECG and hydraulic for i.v. infusions), as described elsewhere⁷. In a second group of five HODI rats, sleep recordings were performed but instead of vasopressin the rats received continuous i.v. infusions of water in an amount equal to their respective daily oral intakes (range 230–320 ml).

As expected, the infusion of vasopressin brought about an almost total disappearance of the spontaneous oral water intake (daily residual intakes being 5–8 ml). Administration of vasopressin resulted in a significant increase (65%) in the daily

duration of PS (Fig. 1). A slight but significant increase (25%) in the total duration of SWS was also observed (Fig. 1). This increase in the duration of sleep was observed both during the day and the night (Table 1). From these results one would suspect that the hereditary absence of vasopressin is the cause of sleep deficits in HODI rats. However, this does not seem to be the case as similar increases of PS and SWS were obtained (Fig. 1 and Table 1) when the total daily water intake was infused i.v., thus inhibiting drinking (residual intakes being 10 to 15 ml) and allowing the rat to be continuously hydrated.

These results clearly show that sleep deficits and particularly the reduction of PS in HODI rats are not due to the lack of vasopressin but more probably to the necessity for these rats to continuously rehydrate themselves. Interestingly, the daily SWS and PS durations returned to pre-infusion levels both in the case of vasopressin and water once the infusions stopped. The fact that the spontaneous rehydration affects only PS could be explained by the elevated number of drinking episodes (once every 5–10 min) which often interrupt SWS but not PS, thus resulting in shorter (and more frequent) SWS phases. Indeed, I have observed (unpublished data) that PS occurrence is closely related to the duration of the preceding SWS episodes. Thus, any shortening of SWS episodes (as was the case for HODI rats) would result in a decrease in the number of PS episodes.

Whatever the origin of sleep deficits in HODI rats in normal conditions, it is important to point out the possible role of PS impairment in the learning ability and memory consolidation of these rats. Indeed, numerous reports have shown that any impairment of this particular stage of sleep in normal animals would bring about an alteration in the acquisition and/or retention of information^{4–6}. Therefore, it seems likely that the reported impairment of learning and memory processes in HODI rats is not due directly to the absence of vasopressin but rather to the decreased amount of PS. The reported restoration of memory function in HODI rats following vasopressin administration¹ could then be attributed not to vasopressin *per se* but to the restoration of normal if not higher levels of PS.

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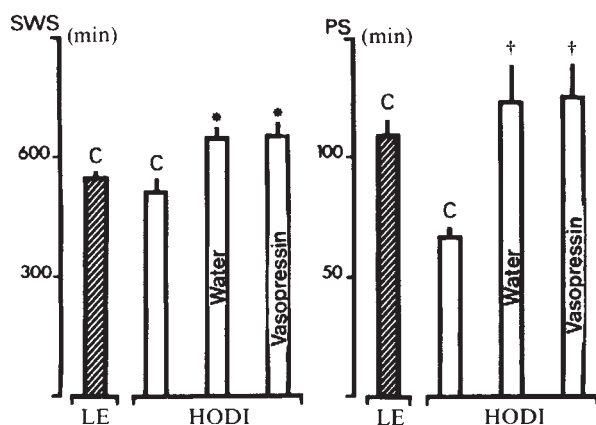


Fig. 1 Mean ($\pm$ s.e.m.) daily durations of slow wave sleep (SWS) and paradoxical sleep (PS) for Long Evans (LE) rats in normal conditions (C) and for homozygous diabetes insipidus (HODI) Brattleboro rats in control conditions (C), following i.v. infusions of the normal daily water intake and after i.v. vasopressin administration. * $P < 0.05$; † $P < 0.001$ in Student's *t*-test, control versus experimental (water or vasopressin) data of HODI rats.

Multiple tumour-specific antigens expressed on a single tumour cell

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Tumours induced by physical or chemical carcinogens often express tumour-specific antigens that can induce strong protective immune defence in the host. The diversity of these unique antigens among different tumours is seemingly endless, and has been compared to that of immune receptors¹. At present, the nature and complexity of this antigenicity is not known for any single tumour. Here we describe the unique antigenicity expressed by a murine ultraviolet light (UV)-induced fibrosarcoma. This tumour is clearly subject to immune surveillance by the normal host², and does not grow progressively unless it undergoes antigenic changes³. Using defined monoclonal T-cell probes and tumour variants selected *in vitro* with these probes, we found that the total antigenicity consisted of multiple independent components, all of which were tumour-specific and expressed simultaneously on the same tumour cell. The demonstration of this antigenic complexity will enable us to identify and compare the molecular composition of the components of this antigen, as well as to determine their individual roles in tumour rejection and escape.

Many UV-induced tumours are highly immunogenic and display tumour-specific antigens on their surface⁴. By selectively suppressing the immune response to the UV-induced 1591 tumour, we have shown that its rejection by normal unimmunized syngeneic hosts is dependent upon T cells specifically reactive with this regressor tumour^{5,6}. In other experiments, we found that tumour-specific T cells consistently failed to recognize variants which grew progressively in normal syngeneic mice³. Both pieces of evidence suggested the significance of a tumour-specific antigen on this regressor tumour recognized by specific T cells. In recent studies, we observed that an immune response could be generated against the regressor variants, and that this response also recognized the parental regressor tumour (J. Urban, unpublished results). Thus, it appeared that the 'antigen' on the 1591 regressor tumour may be complex; in addition to the antigen recognized by the regressor-specific response, it may also contain another antigen that was retained on the regressor variant cells.

We therefore characterized the 1591-specific antigen by using cytolytic T-cell lines specific for 1591 regressor tumour cells and *in vitro*-selected tumour variants. Briefly, a 1591-specific T-cell clone (anti-'A') was used to select for antigen-loss variants of 1591 (1591-A variants). These variants were then used as immunogens to determine if they retained other antigens. Upon finding such a retained antigen ('B'), a second T-cell line (anti-B) was generated which was used to select for loss of the B antigen. By successively continuing this process of immunization followed by selection, a 'C' antigen, then a 'D' antigen were identified. The details of the results are shown in Fig. 1. To confirm that these four T-cell reactivities recognized four different antigens, variants were selected (designated 1591-V1 to 1591-V5) that expressed different combinations of these antigens, thus demonstrating their independence. For example, the independence of the A and B antigens was demonstrated by comparing the phenotypes of 1591-V4 (A⁺B⁺C⁻D⁺) and 1591-V2 (A⁻B⁺C⁻D⁺), for both of these variants lost either the A or B antigen while retaining the other. The independence of the B and C antigens was demonstrated by comparing 1591-V3 (A⁻B⁺C⁺D⁺) and 1591-V2 (A⁻B⁺C⁻D⁺); variants which

had lost either the B or C antigen independently of each other. The A and C antigens were identified as distinct independent structures by the analysis of 1591-V4 (A⁺B⁺C⁻D⁺) and 1591-V3 (A⁻B⁺C⁺D⁺). Lastly, the A, B and C antigens were defined as independent from the D antigen because of the phenotype of 1591-V5 (A⁻B⁻C⁻D⁺).

It appeared that only the appropriate antigen was selectively lost after exposure to each T-cell line. In every instance, the other unique antigens present on the variants before selection were not lost and the expression of other surface antigens, such as H-2K and H-2D, seemed to be not significantly altered by such selections, as determined by cytofluorography with monoclonal antibodies (anti-K^k, 16-3-22; anti-D^k, 15-5-5; ref. 7), using a fluorescence-activated cell sorter (FACS-IV, Becton Dickinson; data not shown). The T cell-selected tumour variants showed a remarkable antigenic stability during continuous passage in culture for 4 months or for four passages in congenic nude mice. The frequency of variants that had lost a single tumour antigen was approximately 10⁻⁴ in a 2-month-old cloned population, and too low to be detected in a freshly cloned population. The simultaneous loss of two antigens from one cell was also too low to be detected, making successive selections necessary to obtain the above variants.

Importantly, the A, B, C and D antigens were all present on the cloned 1591-RE1 regressor tumour line (A⁺B⁺C⁺D⁺), that was derived from the 1591 tumour after the fifth transplant generation in thymectomized, X-irradiated or nude mice. Apparently, except for rare variants such as those described above, every cell in this cloned population expressed all of these four antigens because complete elimination of 1591-RE1 was obtained with T cells having any one of the four T-cell reactivities. Furthermore, as shown in Fig. 1 (far right panel) and Fig. 2, these antigens appeared to be uniquely expressed on cells of the 1591 lineage since they were not found on other UV-induced tumours.

At present, we do not know the genetic origins of the multiple tumour-specific antigens that apparently can be expressed by a single malignant cell, nor if regulatory mechanisms are involved in such antigenic variation. The antigens generating this diversity may or may not belong to a family of related structures. It is unlikely, however, that they represent normal histocompatibility antigens that are recognized by the host because of genetic drift from the strain of origin of the tumour studied. This is improbable because our T-cell probes failed to react with other UV-induced fibrosarcomas which had been induced at the same time, by the same agent, in the same stock of mice as the tumour we have analysed. Although a similar multiplicity of tumour-specific antigens has not yet been described for other tumours, it is interesting that multiple independent tumour-associated antigens can be artificially introduced into a poorly immunogenic tumour by chemical mutagenesis of the malignant cells *in vitro*⁸. We are presently determining if still other antigens exist on the 1591 tumour, which would raise the possibility of an endless variety of unique antigens. African trypanosomes also express an enormous variety of surface antigens, yet upon each reinfection a particular antigen is always initially re-expressed followed by a somewhat predictable sequence of antigenic variation⁹. Thus, although the antigen-loss tumour variants that we describe appear very stable, we cannot yet exclude the possibility that certain environmental influences may cause the reappearance of previous 'lost' antigens or the expression of new antigens. We also do not know whether these multiple unique tumour-specific antigens arose during or subsequent to malignant transformation, nor do we know whether there is a relationship between these antigens and the malignant phenotype of the cells. In any event, knowledge of such antigenic multiplicity may help us to study the mechanism responsible for the generation of diversity of unique antigens expressed by different tumours, as well as the possible function of these antigens.

Of the multiple tumour-specific antigens described above, two (A and C) are involved in rejection of the tumour by the

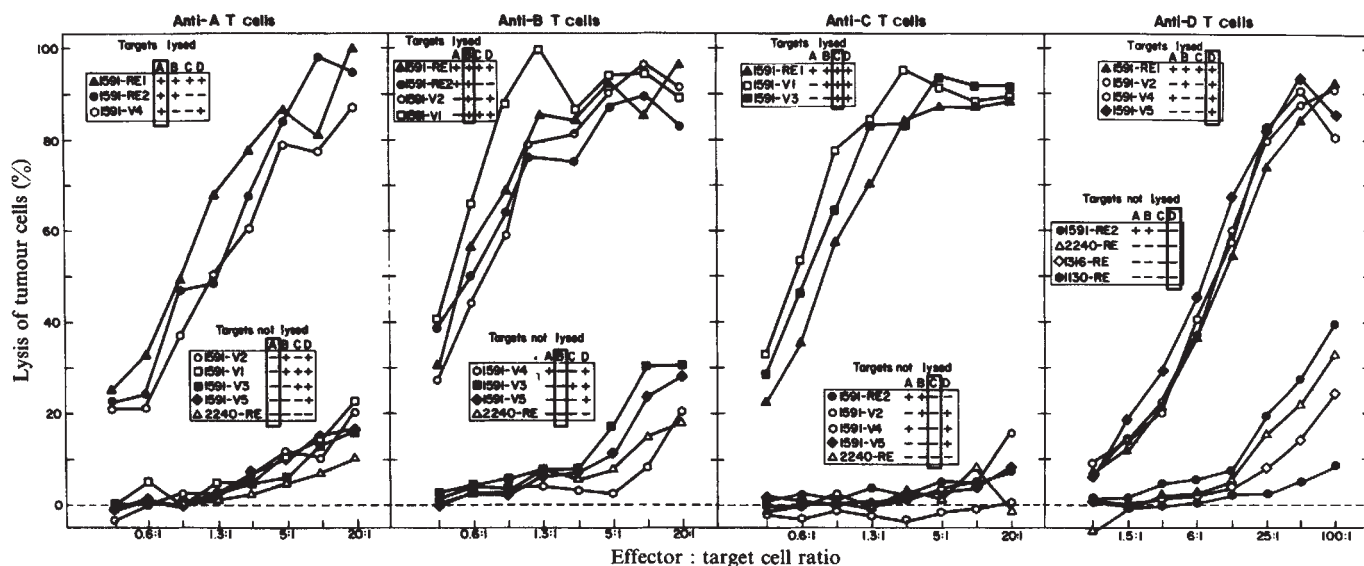


Fig. 1 Multiplicity and independence of 1591-specific antigens as demonstrated by cytolytic T cells and immunoselected variants. Tumour-specific cytolytic T cells were generated in mixed lymphocyte-tumour cell cultures (MLTC) from the spleen cells of syngeneic tumour-immune C3H/HeN mice (mammary tumour virus-negative [MTV⁻] germ-free-derived mice were obtained from the NCI-Frederick Cancer Research Facility Animal Production Area) as previously described³. Briefly, animals were immunized with one injection of 10^7 1591-RE2 cells intraperitoneally (i.p.), or two injections of 10^7 1591-V2, 1591-V1, or 1591-V5 cell (i.p.) (3 weeks apart). Three weeks later, 2.5×10^7 spleen cells from tumour-immune animals were restimulated in a 7-day culture with 2.5×10^5 mitomycin C-treated stimulator cells of the same tumour in 20 ml of RPMI-1640 (Gibco 430-1800) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 5×10^{-5} M 2-mercaptoethanol (CRPMI). Cultures were prepared in upright standing 25-cm² tissue culture flasks (3013; Falcon) and assayed for cytolytic activity on day 7. The anti-A, anti-B and anti-C and anti-D T cells were derived from mice immunized with 1591-RE2, 1591-V2, 1591-V1 or 1591-V5 respectively. The anti-A, anti-B and anti-C T cells were then established as long-term T-cell lines using a method described elsewhere in detail¹⁰. Briefly, the 7-day-old MLTC cells were washed once, resuspended in CRPMI and then placed in conical microculture wells (76-224-05, Linbro) at 10^3 cells per well in 0.2 ml medium. Each well contained 1×10^6 irradiated (2,000 R) syngeneic spleen cells, 500 mitomycin C-treated tumour cells, and 33% (v/v) T-cell growth factor (derived from a secondary mixed lymphocyte culture¹¹). After 6-8 days, cultures were washed once and passed at 10^3 cells per well using the same conditions outlined above. The anti-A, anti-B and anti-C T-cell lines demonstrated distinct cytolytic reactivity patterns. The lines were subsequently cloned by limiting dilution. Antigen-loss variants were selected *in vitro* by exposing tumour cells to the cytolytic T cells as previously described¹⁰. Briefly, 10^2 to 10^4 tumour cells were added to flat-bottom microculture wells (76-003-05, Linbro) in 100 μ l minimal essential medium containing 10% fetal bovine serum and 1% penicillin-streptomycin (CMEM) and incubated at 37°C for 4-6 h to allow fibroblasts to attach. 10^5 Cytolytic T cells were then added to each well in an additional 100 μ l of CMEM; 24 h later, wells were gently washed to remove most of the T cells and detached killed tumour cells. After 2 weeks, colonies of tumour cells which had survived the first killing were then retreated with T cells. Tumour cells which survived this second killing were then cloned, expanded and cryopreserved. All selections were made using either the cloned anti-A, the uncloned anti-B or uncloned anti-C T-cell lines. The antigen-loss variants that are presented here have been arbitrarily designated as 1591-V1 to 1591-V5; 1591-V1 (A⁺B⁺C⁺D⁺), 1591-V2 (A⁺B⁺C⁺D⁺), 1591-V3 (A⁺B⁺C⁺D⁺), 1591-V4 (A⁺B⁺C⁺D⁺) and 1591-V5 (A⁺B⁺C⁺D⁺). The cytolytic reactivity of the cloned anti-A, anti-B or anti-C T-cell lines, or the anti-D MLTC cells were tested in a 4.5 h ⁵¹Cr release assay against the indicated targets. This was performed by serially diluting the effector cells in V-bottom wells in 100 μ l CMEM into which 5×10^3 target cells, labelled with ⁵¹Cr for 45 min at 37°C, were added in 100 μ l CMEM. Plates were spun at 450g for 3 min and after 4.5 h of incubation, 100 μ l of supernatant medium were analysed for radioactivity using a γ counter. The % specific lysis was calculated by the formula:

$$\% \text{ specific lysis} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100$$

Spontaneous release was <15% of the total release of radiolabel. The derivation of 1591-RE1 is explained in the text and 1591-RE2 is an independent *in vitro* adaptation of the 1591-regressor tumour. 2240-RE, 1316-RE and 1130-RE are described in Fig. 2. Variants 1591-V1 and 1591-V3 expressed the D antigen but were not included as targets of the anti-D T cells in this figure.

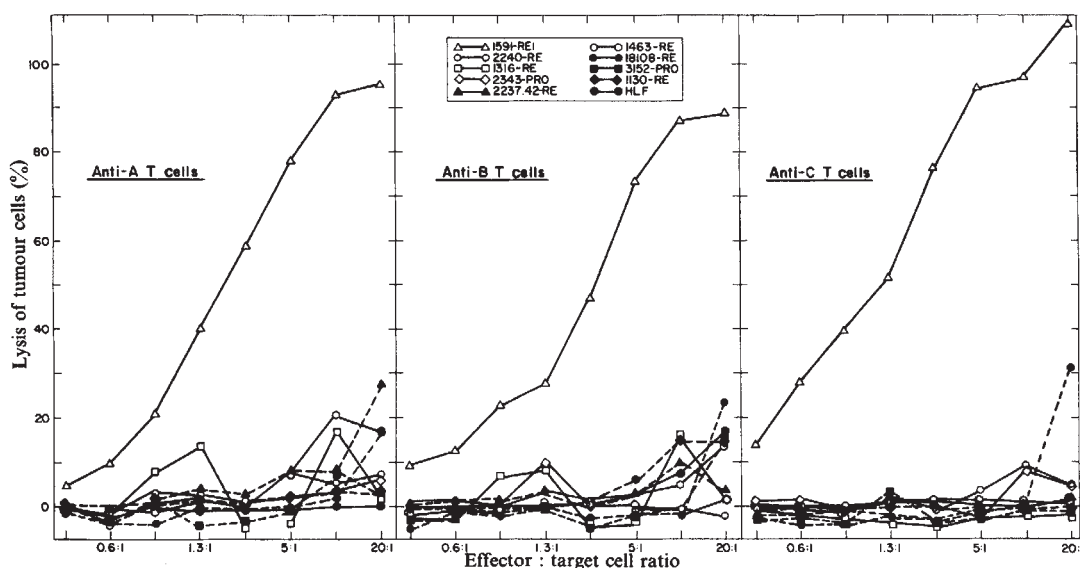


Fig. 2 Unique specificity of tumour-reactive T cells for 1591 tumour cells. The cytolytic reactivity pattern of the cloned anti-A, anti-B and anti-C T-cell lines was determined in a 4.5 h ⁵¹Cr release assay against the indicated targets. 1591-RE, 2240-RE, 1316-RE, 1463-RE, 18108-RE, 2237-RE and 2343-PRO are UV-induced fibrosarcomas that were derived from C3H/HeN (MTV⁻) mice at the NCI-Frederick Cancer Research Facility. The suffix RE or PRO stands for a regressor or progressor phenotype, respectively. Tumours 1130-RE and 3152-PRO are fibrosarcomas that were induced in C3H/HeN (MTV⁻) mice by the subcutaneous injection of 3-methylcholanthrene. HLF is a nonmalignant, nontransformed fibroblast line derived from heart and lung tissue of an adult C3H/HeN (MTV⁻) mouse.

normal host (R.D.W., unpublished results), while the *in vivo* significance of the other antigens is currently being investigated. It is curious that when these multiple antigens are simultaneously expressed on the tumour cell, they are not all recognized by the host at the same time¹⁰. This 'hierarchy' in the host recognition of these antigens may make tumour progression more likely since fewer antigens would have to be lost for effective immune escape. However, such hierarchy would also lead to the retention of certain antigens on progressor variants, as we find in the described tumour system, thus making these retained antigens uniquely suited as target structures for passive immunotherapy, and therefore clinically relevant. In addition, because multiple tumour-specific antigens remained on these progressor variants, the simultaneous use of immunological probes directed against several of these independent target structures will greatly reduce the probability of immune escape from such immunotherapy.

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Homology between phosphotyrosine acceptor site of human *c-abl* and viral oncogene products

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The human homologues of several independent viral oncogenes, each of which encodes tyrosine-specific protein kinases, have been identified^{1,2}. Of these, three (*v-src*, *v-yes* and *v-fes/fps*) are known to exhibit considerable sequence homology, particularly in the regions of their phosphorylation acceptor sites³⁻⁵. In the present study, sequences encoding the tyrosine phosphorylation acceptor sites of the Abelson murine leukaemia virus oncogene, *v-abl*, and its human cellular homologue, *c-abl*, have been identified and their nucleic acid sequences determined. Our results establish extensive homology between this region of *c-abl* and acceptor domains of the *v-src*^{3,6}, *v-yes*⁶ and *v-fes/fps*^{4,5} family of viral oncogenes, as well as more distant relatedness to the catalytic chain of the mammalian cyclic AMP-dependent protein kinase⁷. These findings suggest that, of the homologues of retroviral oncogenes with tyrosine protein kinase activity examined to date, all were probably derived from a common progenitor and may represent members of a diverse family of cellular protein kinases.

We have described previously the application of a cosmid vector system to the molecular cloning of the human cellular homologue of acquired cellular (*v-abl*) sequences of the

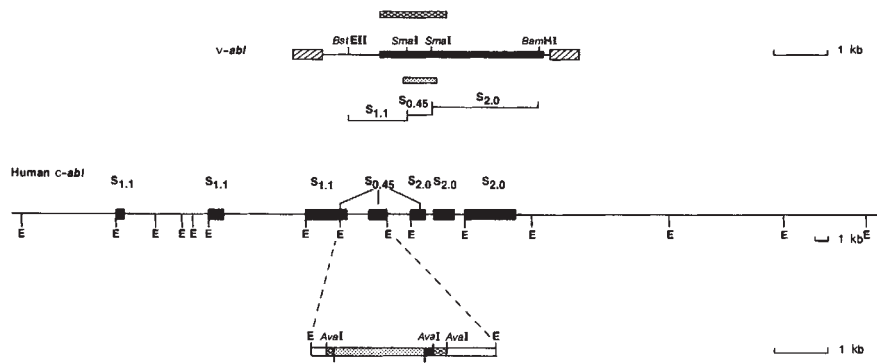
Abelson strain of murine leukaemia virus⁸. *v-abl* homologous sequences were found to encompass six separate coding regions (exons) distributed over a total of 32 kilobases (kb)⁸ localized on human chromosome 9(q34) (refs 9, 10). Figure 1 shows the positioning of such sequences, as delineated by restriction endonuclease and hybridization analysis. Hybridization of individual exons to a series of *SmaI* restriction fragments corresponding to specific regions of *v-abl* is also indicated. By transfection analysis, *v-abl*-specific sequences required for transforming activity have been localized to the 5' half of the acquired region within the viral genome¹¹. Moreover, a variant of Abelson-MuLV characterized by an in-phase interstitial deletion mapping within the 5' region of the *v-abl* acquired sequence has been shown to lack transforming activity¹². Since this latter deletion approximates that of a 0.45-kb *SmaI* restriction fragment of *v-abl*, shown in Fig. 1, we reasoned that human *c-abl* sequences homologous to the *S*_{0.45} probe might correspond to its phosphotyrosine acceptor site. As shown in Fig. 1, most of the *S*_{0.45} homologous sequences could be localized to a single 3.4-kb *EcoRI* restriction fragment encompassing portions of three exons and mapping within the middle of the human *v-abl* homologous region. Subcloning in pBR328 and fine mapping revealed that this fragment contained a 0.14-kb *BglII/AvaI* restriction fragment which hybridized strongly to the *S*_{0.45} probe.

In view of its homology to the transformation-specific region of *v-abl* and consequently the possibility that it might correspond to the active region of the *c-abl* protein kinase, the 0.14-kb *BglII/AvaI* restriction fragment was subjected to nucleic acid sequence analysis. Figure 2 shows that of the three possible reading frames, the first contained six termination codons, the second contained a single TGA stop codon near the middle of the sequence, not followed by any obvious splice acceptor site, while the third lacked any termination codons and thus appeared to represent the authentic coding sequence for the *c-abl* gene product. To extend this analysis, we subsequently sequenced the coding region of an adjacent downstream *AvaI/AvaI* *c-abl* restriction fragment, and by an analogous approach, the corresponding region of *v-abl*. The predicted amino acid sequences corresponding to each of these regions of nucleic acid sequence are shown in Fig. 2. The predicted amino acid sequences of the internal 61 residues were identical based on either the *v-abl* or *c-abl* nucleic acid sequence. Sixteen additional amino-terminal and 18 carboxy-terminal residues correspond to regions for which only *v-abl* sequence was determined.

We investigated whether the deduced *c-abl/v-abl* amino acid sequence corresponds to phosphotyrosine acceptor site regions common to the *v-src*-, *v-yes*- and *v-fes/fps*-encoded transforming proteins by comparing relevant sequences. As shown in Fig. 3, the *c-abl/v-abl* sequence contains 38 out of 96 residues in common with all four of the viral acceptor site regions examined. Additional relatedness with individual viral sequences appears rather random; some *c-abl/v-abl* residues are common to either *v-yes* or *v-src* while others are shared only with the *v-fes* and *v-fps* gene products. The *c-abl/v-abl*-encoded proteins also contain a tyrosine residue at the position corresponding to the previously identified tyrosine acceptor sites of each of the other oncogene-encoded products examined. In addition, there is considerable homology (28 of 96 residues) between *c-abl* and the catalytic subunit of the mammalian cyclic AMP-dependent protein kinase (BOV-PK), although the tyrosine acceptor residue of *c-abl/v-abl* is replaced by a tryptophan residue in BOV-PK. Interestingly, this substitution is directly adjacent to a threonine residue known to represent one of two phosphorylation sites previously identified in BOV-PK⁷.

To establish whether the putative *c-abl* tyrosine phosphorylation site is recognized by functionally active viral protein kinases, a synthetic peptide with the sequence NH₂-Ser-Arg-Leu-Met-Thr-Gly-Asp-Thr-Tyr-Thr-Ala-His-Ala-Gly-Ala-Lys-COOH was chemically synthesized and purified by HPLC.

Fig. 1 Schematic representation of Abelson-MuLV proviral DNA and the human *v-abl* homologous (*c-abl*) sequence. Positions of long terminal repeats (▨), acquired cellular sequences (■), region of the acquired sequences necessary to retain transforming activity (▤), a deletion within a transformation-defective variant of Abelson MuLV (□), and a series of three *Sma*I restriction fragments (▢) used as probes for analysis of *c-abl* sequences, are shown in the upper portion of the figure. *v-abl* homologous sequences, shown as solid boxes in the human *c-abl* genetic locus, were delineated by restriction endonuclease digestion and molecular hybridization analysis as described previously⁸. Specific *v-abl* probes, to which the individual *c-abl* exons hybridize, are also shown. A restriction map of the phosphotyrosine acceptor site region of *c-abl* is shown in more detail directly below the *c-abl* map; extents of hybridization to the *S*_{0.45} probe are indicated as negative (□), weak (▤), medium (▤), or strong (■).



ile ser ser ala met glu tyr leu glu lys lys asn phe ile his arg
v-abl 1 ATC TCA TCA GCC ATG GAG TAC TTG GAG AAG AAG AAC TTC ATC CAC AGA
 Bp11
 asp leu ala ala arg asn cys leu val gly glu asn his leu val lys
v-abl 49 GAC CTT GCT GCC CGG AAC TGC CTG GTA GGG GAA AAC CAC TTG GTG AAG
c-abl GAT CTT GCT GCC CGA AAC TGC CTG GTA GGG GAG AAC CAC TTG GTG AAG
 Bp11
 val ala asp phe gly leu ser arg leu met thr gly asp thr tyr thr
v-abl 97 GTG GCT GAT TTT GGC CTG AGC AGG TTG ATG ACA GGG GAC ACC TAC ACG
c-abl GTA GCT GAT TTT GGC CTG AGC AGG TTG ATG ACA GGG GAC ACC TAC ACA
 ala his ala gly ala lys phe pro ile lys trp thr ala pro glu ser
v-abl 145 GCC CAT GCT GGA GCC AAA TTC CCC ATC AAA TGG ACC GCA CCT GAG AGC
c-abl GCC CAT GCT GGA GCC AAG TTC CCC ATC AAA TGG ACT GCA CCG GAG AGC
 Ava1
 leu ala tyr asn lys phe ser ile lys ser asp val trp ala phe gly
v-abl 193 CTA GCC TAC AAC AAG TTC TCC ATC AAG TCG GAC GTG TGG GCA TTT GGA
c-abl CTG GCC TAC AAC AAG TTC TCC ATC AAG TCC GAC GTC TGG GGT AAG GCG
 Putative splice donor
 val leu leu trp glu ile ala thr tyr gly met ser pro tyr pro
v-abl 241 GTA TTG CTC TGG GAG ATT GCT ACC TAT GGC ATG TCA CCT TAC CCG G
 Ava1 Sma1

Fig. 2 Nucleic acid sequence and predicted amino acids of the *v-abl* and human *c-abl* phosphotyrosine acceptor site regions. Both strands of appropriate restriction fragments were labelled with [γ -³²P]ATP and sequenced according to the method of Maxam and Gilbert²⁸.

c-abl/v-abl 10 20 30 40
 I S S A M E Y L E K K N F I H R D L A A R N C L V G E N H L V K V A D F G L S R L M T G D T Y T
*v-fes*ST A A G M E Y L E S K C C I H R D L A A R N C L V T E K N V L K I S D F G M S R E E A D G V Y A
v-fps A A G M E Y L E S K H C I H R D L A A R N C L V T E K N L K I S D F G M S R E E D G V Y A
v-src I A S G M A Y I E R M N Y I H R D L A A R N C L V G E N L V K V A D F G L A R L I E D N E Y T
v-yes I A D G M A Y I E R M N Y I H R D L A A R N C L V G D N L V C K I A D F G L A R L I E D N E Y T
 BOV-PK I V L T F E Y L S L D L I Y R D L K P E N L I D Q Q G Y I Q V T D F G F A K R V K G R T W T
 I E Y L I R D L N L V D F G G T T
 50 60 70 80 90
c-abl/v-abl A H A G A K F P I K W T A P E S L A Y N K F S I K S D V W A F G V L L W E I A T Y G M S P Y P
*v-fes*ST A S G G L R L P V K W T A P E A L N Y G R Y S S E S D V W S F G I L L W E T F S L G A S P Y P
v-fps S T G G M K Q I P V K W T A P E A L N Y G W Y S S E S D V W S F G I L L W E A F S L G A V P Y A
v-src A R Q G A K F P I K W T A P E A A L Y G R F T I K S D V W S F G I L L T E L T T K G R V P Y P
v-yes A R Q G A K F P I K W T A P E A A L Y G R F T I K S D V W S F G I L L T E L T V T K G R V P Y P
 BOV-PK L C G T P E - - - - Y L A P E I I L S K G Y N K A V D W W A L G V L I Y E M A G Y P P F F A
 A P E D W A G V L E A

Fig. 3 Comparison of the deduced amino acid sequence of the *v-abl* and human *c-abl* phosphotyrosine acceptor sites to analogous regions of the *v-fes*ST (ref. 4), *v-fps*⁵, *v-src*⁶ and *v-yes*⁶ encoded transforming proteins and to the catalytic subunit of the mammalian cyclic AMP-dependent protein kinase⁷. Amino acids common to each of the oncogene-encoded acceptor domains are shown in open boxes, while those residues shared by the *c-abl*-encoded sequence and BOV-PK are indicated in the lower portion of the figure. The position of the putative phosphotyrosine acceptor residue (*) is also shown. A, alanine; G, glycine; T, threonine; C, cysteine; P, proline; M, methionine; V, valine; I, isoleucine; L, leucine; Y, tyrosine; F, phenylalanine; W, tryptophan; D, aspartic acid; E, glutamic acid; R, arginine; K, lysine; H, histidine; S, serine; N, asparagine; Q, glutamine.

On addition of Abelson-MuLV P120 to immunoprecipitates, the *c-abl* acceptor peptide was phosphorylated 10-fold more efficiently than in the same reaction conditions using [Val⁵]angiotensin II as substrate¹³. Following re-purification of the labelled peptide by HPLC and subsequent two-dimensional phosphoamino acid analysis, ³²P incorporation by the synthetic *c-abl* acceptor site peptide was found to be exclusively in tyrosine.

Table 1 summarizes the relatedness of the amino acid sequences within the acceptor regions of representative tyrosine-specific protein kinases and the corresponding nucleic acid sequences from which these were deduced. Although *c-abl* is somewhat more related to *v-src*, especially with respect to nucleic acid sequence, than to the other viral oncogene sequences examined, it is less related to *v-src* than is *v-yes*. Interestingly, of the five oncogene acceptor sequences analysed, *c-abl* is most closely related to BOV-PK. It is also apparent that, in general, the oncogene acceptor site regions are much less conserved with respect to nucleic acid sequence than in the amino acids they encode, indicating that much of the nucleic acid sequence divergence reflects third position or other silent nucleotide changes not resulting in an altered gene product.

The present findings establish the evolutionary relatedness of *c-abl/v-abl* to four retroviral oncogenes, each of which contains transformation-specific sequences with tyrosine-specific protein kinase activity. In fact, despite its human cellular origin, the

phosphotyrosine acceptor site region of *c-abl* is as closely related to analogous regions of the *v-src*, *v-yes* and *v-fes/v-fps* viral oncogenes as these are to each other. Although an additional retroviral oncogene, *v-ros*, with similar enzymatic activity has been described, its relationship to those genes examined in the present study is unknown¹⁴. Human *c-abl*, *c-src* and *c-fes/c-fps* are sufficiently diverged to lack detectable cross-hybridizing sequences and have been mapped to different human chromosomes^{9,10,15,16}. The mammalian *v-fes* and avian *v-fps* genes, although independently identified, correspond to a common mammalian cellular genetic locus, *c-fes/c-fps*^{17,18}. The human homologue of the fourth viral oncogene, *v-yes*, remains poorly defined.

Evidence that the *c-abl* phosphotyrosine acceptor site identified in the present study is functionally active derives from its *in vitro* phosphorylation by the *v-abl* protein kinase. Moreover, the identity of this site to that of *v-abl*, and its close resemblance to the well characterized *v-src*, *v-yes* and *v-fes/v-fps* tyrosine phosphorylation acceptor sites strongly favours the possibility that this site has functional activity. The major *in vitro* and *in vivo* phosphorylated tyrosine residue of *v-abl* has been localized seven residues distal to a trypsin cleavage site^{19,20}, consistent with the positioning of the *v-abl* and human *c-abl* tyrosines seven amino acids distal to arginine residues. An analogous approach was first used to identify the acceptor sites common to the *v-src*, *v-yes* and *v-fes/v-fps* gene products²¹⁻²⁴.

Table 1 Segment comparison scores of human *c-abl* and representative viral oncogene phosphotyrosine acceptor sites and BOV-PK

Sequence	<i>c-abl</i>	<i>v-fes</i>	SD units		
			<i>v-fps</i>	<i>v-src</i>	<i>v-yes</i>
<i>c-abl</i>	—	—	—	—	—
<i>v-fes</i>	17.6(6.7)	—	—	—	—
<i>v-fps</i>	15.6(7.8)	19.5(16.9)	—	—	—
<i>v-src</i>	18.9(11.4)	11.9(3.2)	13.6(3.0)	—	—
<i>v-yes</i>	17.1(5.2)	15.7(0.2)	9.8(1.6)	42.5(15.8)	—
BOV-PK	7.6(—)	5.4(—)	4.8(—)	5.7(—)	4.5(—)

Extents of relatedness of amino acid sequences designated 17–64 in Fig. 3 and corresponding nucleic acid sequences from which they were derived. Results are expressed in SD units based on 20-residue segment lengths according to the Relate program as described by Barker and Dayhoff²⁷. A score of >5 SD indicates evolutionary relatedness of two proteins and scores between 3 and 5 SD support relationship if there are other indications such as a similarity of function²⁷.

Although the requirement for a functionally active tyrosine-specific protein kinase for *v-abl*-associated transformation has been demonstrated using transformation-defective mutants^{25,26}, the involvement of the major tyrosine acceptor in this process remains to be determined.

The conservation of phosphotyrosine acceptor sequences throughout vertebrate evolution, and the finding that most of the observed divergence between different genes encoding such sequences involves silent base substitutions, argues that each confers on its host some essential biological function. Presumably, divergence of these sequences occurred early in vertebrate evolution before separation of avian and mammalian classes. The possible involvement of tyrosine-specific cellular protein kinases in human cancer remains to be determined. However, the high frequency with which recombinant transforming viruses containing such sequences have been isolated and the recent localization of *c-abl* within a small fragment of human chromosome 9 which is translocated to chromosome 22 in chronic myelogenous leukaemia^{9,10}, would seem to favour such a model.

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Chromosomal sublocalization of human *c-myb* and *c-fes* cellular *onc* genes

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The transforming genes of acutely transforming retroviruses are derived from conserved cellular genes (*c-onc* genes) which are believed to be important in normal cell growth and differentiation^{1–4}. Recent studies indicate that altered expression of *c-onc* genes, for example, by insertion of viral genomes^{5,6}, gene amplification^{7,8} or chromosomal translocation^{9–12}, can lead to development of malignant diseases in man and animals. *c-myb* and *c-fes* are homologues of the transforming genes of avian myeloblastosis virus and feline sarcoma virus (Gardner and Snyder-Theilen strains), respectively. *c-myb* is transcribed preferentially in immature haematopoietic cells¹³ and probably codes for a protein important in differentiation of these cells. The viral *fes* gene, like several other viral *onc* genes, encodes a tyrosine-specific protein kinase¹⁴. However, *c-fes* transcripts have not been detected in the types of human cells examined so far^{15,16}. *c-myb* and *c-fes* have been assigned to human chromosomes 6 and 15, respectively^{17,18}. Specific aberrations involving these chromosomes have been observed at high frequency in several human neoplasms¹⁹. We have now sub-localized *c-myb* to 6q22–24 and *c-fes* to 15q25–26 by *in situ* hybridization of the human *c-onc* probes to human mitotic chromosome preparations^{20,21}. These chromosomal segments are indeed involved in nonrandom translocations in several human tumours. The results encourage further investigation into the role of *onc* genes in the pathogenesis of specific neoplasms.

In situ hybridization of the ³H-labelled *c-myb* probe, plasmid pF8 (ref. 22), resulted in significant label on the long arm of chromosome 6, slightly distal to the mid-portion of the arm. Figure 1a illustrates the compiled distribution of labelled sites (one to four grains each) in 163 cells represented as a histogram along the human haploid karyotype. Of the 163 cells (30.7%), 50 exhibited label on region q2 of one or both chromosomes 6; these sites represented 7.4% (51/685) of all labelled sites throughout the 163 cells. Although highly significant, these compiled results from several experiments indicated relatively high nonspecific probe binding. However, analysis of one slide showed that 45.1% of cells (23/51) exhibited grains on one or both chromosomes 6 on region q2. Of 216 labelled sites, 21 (9.7%) were located on 6q2, as illustrated by the representative metaphase cell in Fig. 2b. Compilation of data from a large number of labelled chromosomes 6 (88) confirmed the hybridization to 6q2 and also indicated significant clustering of grains to bands q22–24 (52.1%) (Fig. 1c).

Hybridization of the human *c-fes* probe pN26 (ref. 23) resulted in typical labelling of the distal end of the long arm of chromosome 15. An analysis of 119 cells from several experiments (Fig. 2a) showed that 44 cells (37.0%) exhibited label on region q2 of one or both chromosomes 15. These sites represented 12.2% (48/393) of all labelled sites in the cells analysed. The most significant hybridization in one experiment resulted in 55.8% (24/43) of cells showing label on 15q2; these grains represented 21.1% of all labelled sites. Typical labelling of the distal end of the long arm of chromosome 15 is illustrated in Fig. 2b. Compilation of grain positions from 74 labelled chromosomes 15 indicated that 75.7% of labelled sites were

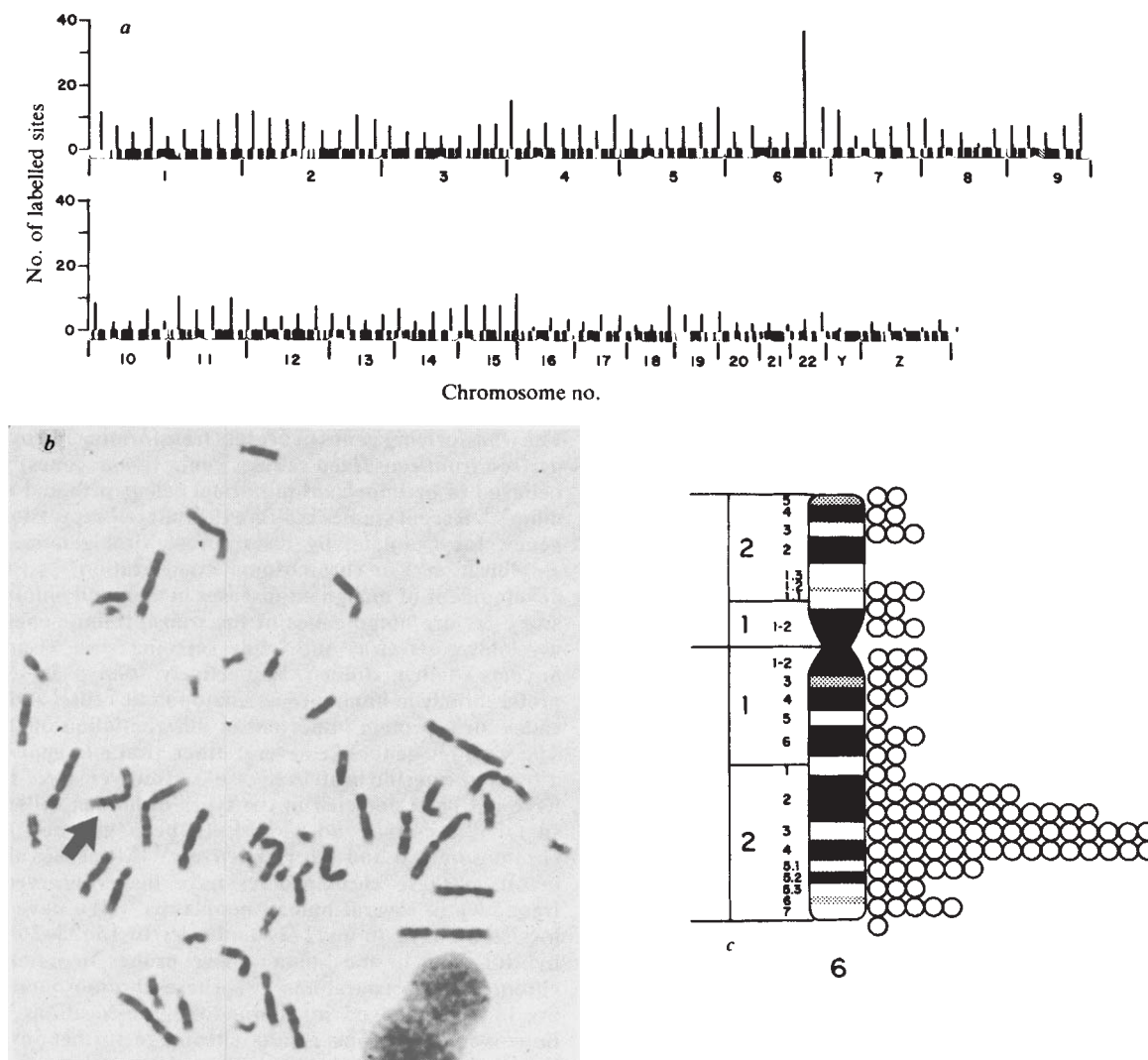


Fig. 1 Sublocalization of the human *c-myb* gene. *a*, Distribution of labelled sites in 163 hybridized cells showing significant clustering of grains on region q2 of chromosome 6. In this analysis, the human haploid karyotype (including X and Y chromosomes) was divided into 110 equal segments and the number of labelled sites plotted for each segment. *b*, Human metaphase cell hybridized with ^3H -labelled pF8, which contains a 2-kilobase (kb) *c-myb*-specific insert²², illustrating typical labelling of 6q22–24 (arrow). Cell was hybridized at 10 ng ml^{-1} for 12 h and exposed to autoradiographic emulsion for 19 days. *c*, Distribution of labelled sites on 88 labelled chromosomes 6. Of 96 total sites, 50 (52.1%) were located on q22–24. pF8 plasmid DNA was ^3H -labelled by nick-translation using ^3H -dATP (51.1 Ci mmol^{-1}) and ^3H -TTP (87.5 Ci mmol^{-1}) (NEN)³⁷ to specific activity $15.6 \times 10^6\text{ c.p.m. per } \mu\text{g}$. DNA was separated from unincorporated ^3H -dNTPs by Sephadex G-75 chromatography. Human mitotic chromosome preparations were obtained from methotrexate-synchronized peripheral blood lymphocyte cultures, hybridized *in situ* with probe at $10\text{--}25\text{ mg ml}^{-1}$, autoradiographed for 6–19 days and G-banded as previously described^{20,21}.

located on segment 15q25–26 (Fig. 2c). The observation that a large percentage of labelled sites (47/74 or 63.5%) were clustered at the end of the long arm suggests that the *c-fes* gene is probably located in band q26.

Consistent chromosomal aberrations are observed in certain human cancers, in particular the haematological neoplasias^{24–26}. Recently, several *c-onc* genes, including *c-myc*^{11,12}, *c-abl*¹⁸, *c-sis*^{27,28} and *c-mos*²⁹, have been regionally mapped to chromosome segments involved in these rearrangements. In fact, the *c-myc* gene was found to be translocated from 8q24 to a site adjacent to the immunoglobulin heavy-chain locus on the 14q+ chromosome in Burkitt's lymphoma^{11,12}. Also, in one case of chronic myelogenous leukaemia, *c-abl* was found to be translocated from chromosome 9 to the 22q+ chromosome (Philadelphia chromosome)³⁰.

In this study, we have regionally mapped two *c-onc* genes, *c-myb* and *c-fes*, in the normal human karyotype using the direct mapping technique of *in situ* hybridization. The *c-myb* gene was found to be located on the long arm of chromosome 6 within bands q22–24. Deletion or translocation of the distal

half of 6q has been consistently observed in acute lymphocytic leukaemia (ALL), malignant lymphoma, ovarian carcinoma and melanoma¹⁹. Specifically, a high percentage of ALL cells exhibit a deletion in bands 6q21–qter^{31,32}. Interestingly, high levels of *c-myb* RNA have been detected in primary ALL cells, as well as in two cell lines (Molt-4 and CEM) established from ALL patients¹³. Molt-4 cells also exhibit³³ a deletion/translocation of the q21–qter segment of one chromosome⁶. Similarly, the break in chromosome 6 in the 6;14 translocation in ovarian carcinoma has been assigned^{34,35} to band q21. It would be of interest to examine tumour cells with the 6q- marker directly by *in situ* hybridization to determine whether *c-myb* has been translocated to another chromosomal location. These studies should be carried out in conjunction with analyses on quantitative and qualitative changes in the expression of *c-myb* that may result.

The human *c-fes* gene was localized to the distal end of the long arm of chromosome 15 within bands q25–26. The distal half of this chromosome (q22–qter) is involved in a reciprocal translocation with 17q21–qter in a high percentage of patients

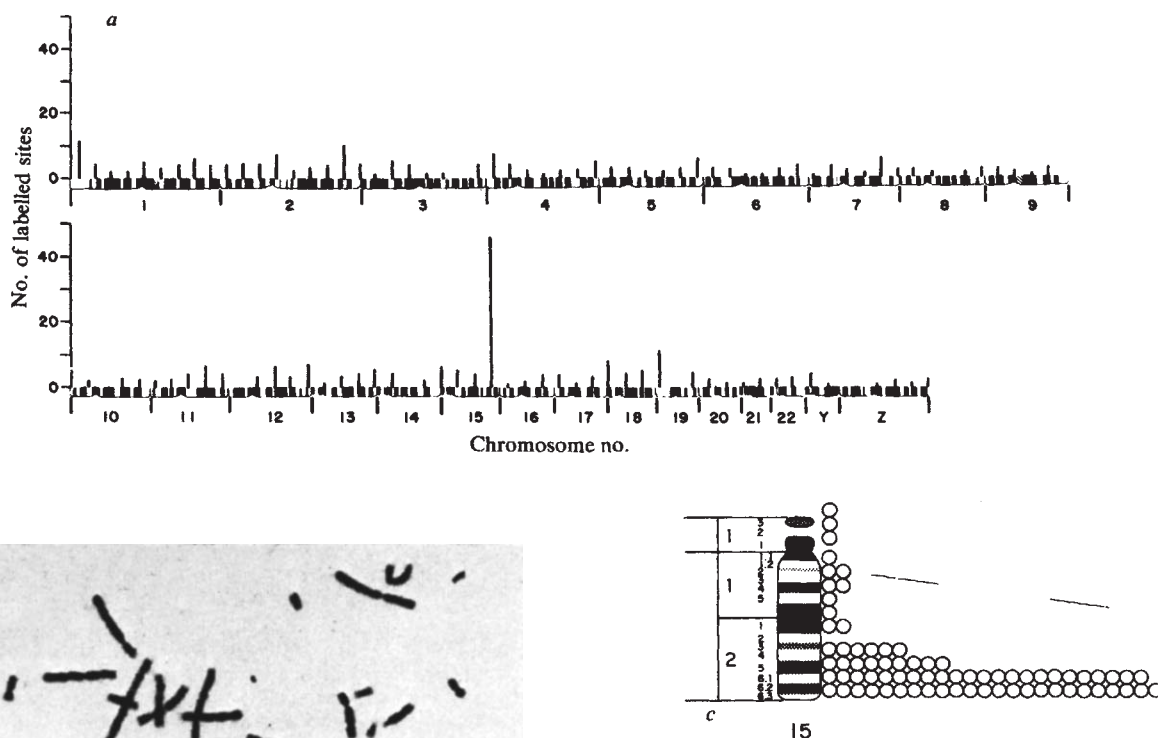


Fig. 2 Sublocalization of the human *c-fes* gene. *a*, Compiled data from 119 cells illustrating significant hybridization to region q2 of chromosome 15. Distribution of label is represented as a histogram along the human haploid karyotype (X and Y chromosomes included) divided by length into 110 equal segments. *b*, Human chromosome spread hybridized with ^3H -labelled pN26, which contains a 4-kb *c-fes*-specific insert²³, showing typical labelling of the distal end of the long arm of chromosome 15 (arrow). The cell was hybridized at $100\ \mu\text{g ml}^{-1}$ for 12 h and exposed for 6 days. *c*, Distribution of grains on 74 labelled chromosomes 15. Of 74 labelled sites, 56 (75.7%) were clustered on q25–26. pN26 plasmid DNA was ^3H -labelled to 21.5×10^6 c.p.m. per μg , chromatographed on Sephadex G-75 and then further purified through NACS-52 (Bethesda Research Laboratories) to reduced nonspecific binding of probe. DNA was hybridized at 25 or $100\ \text{ng ml}^{-1}$, followed by 5–35 days exposure.

with acute promyelocytic leukaemia³⁶. However, the break point in chromosome 15 in this translocation is poorly defined. Again, more experiments are needed to determine the role, if any, of *c-fes* in the pathogenesis of this form of leukaemia.

It has become increasingly apparent that specific chromosomal translocations may be important in some human and animal cancers. These translocations involve cellular genes that may or may not be homologues of retroviral *onc* genes. The *in situ* hybridization technique allows direct examination of the position of any given cellular sequence in normal and transformed cells and may reveal translocations not detected by standard karyotype analysis. In conjunction with studies on the mode of expression of these genes, it should be possible to define the role of potentially oncogenic sequences and their mechanism of activation in the development of cancer.

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Variable amplification of immunoglobulin λ light-chain genes in human populations

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The human λ immunoglobulin locus displays a series of restriction fragment length polymorphisms that are readily detected in small populations of normal individuals¹. Similar polymorphisms appear in populations of wild mice, suggesting that the λ locus¹⁻⁵ is subject to rapid variation within a single species⁶. Here we show that the polymorphisms seen in the human λ locus seem to have arisen from unequal meiotic crossing over, altering the number of λ genes from as few as six to as many as nine per haploid genome. This expansion and contraction in the number of human λ genes is significant in that it may affect an individual's capacity to produce variation among λ light chain genes.

The human λ polymorphisms appear as *Eco*RI restriction fragment length variations. As shown in Fig. 1A, hybridization of *Eco*RI-digested genomic DNA from six normal individuals to a λ constant region (C_λ) probe reveals several different patterns. For example, there is an 8-kilobase (kb) fragment in lanes a-d that is absent in lanes e and f. Similarly, an 18-kb fragment in lanes c, e and f is absent in lanes a, b and d. Fragments at 14 and 16 kb (which contain highly homologous C_λ genes) are found in every individual thus far examined and have been mapped to the 5'- and 3'-most regions of the C_λ locus, respectively (see map, Fig. 1B)¹. Another *Eco*RI DNA fragment common to all individuals examined is 5 kb long and represents a C_λ pseudogene located on a different chromosome from that of the active genes⁷. The location of the polymorphic fragments within the locus is clearly demonstrated when a fragment corresponding to the region between the second and third λ genes of the locus, an intervening sequence (see map, Fig. 1B), is used as a probe (λ IVS) and hybridized to *Eco*RI-digested genomic DNA (Fig. 1A, lanes g-l). Using this probe, 8-, 13-, 18- and 23-kb fragments are easily identified in different individuals. At most, two fragments are present in any one individual, suggesting that the fragments are allelic (see below).

The 13-, 18- and 8-kb fragments do, in fact, segregate in an allelic fashion in two family studies (data not shown). For purposes of this discussion, we assume that the 23-kb fragment is also a discrete allele. Population studies in which 110 genomic DNAs were examined show that the allelic frequencies are the following: 8 kb, 0.75; 13 kb, 0.05; 18 kb, 0.196; 23 kb, 0.01.

The relationship between these polymorphisms and the instability exhibited by these gene segments when cloned in the bacteriophage λ is illustrated by the behaviour of several cloned fragments¹. Purified 13- and 18-kb *Eco*RI fragments were cloned from the genomic DNA of an individual with the 13/18 allotype by selecting clones which hybridized to the λ IVS probe (see Fig. 1B). When these λ CH4A hybrid phage were grown in *recA*⁺ *Escherichia coli*, they gave rise to subpopulations of phage that had undergone characteristic deletions. These subpopulations were separated by CsCl equilibrium density gradients and further characterized by digesting the phage DNA with *Eco*RI to determine insert size. Thus, the clone originally

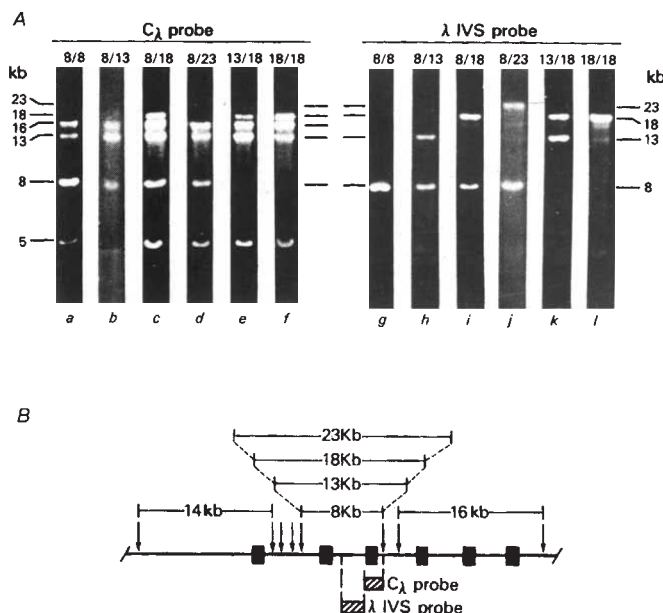


Fig. 1 A, Demonstration of various λ constant region (C_λ) allotypes. *Eco*RI-digested genomic DNA prepared from white blood cells¹ of normal individuals was electrophoresed on 0.8% agarose gels, transferred to nitrocellulose paper¹¹ and hybridized to a C_λ probe in lanes a-f and to a λ IVS probe in lanes g-l. The C_λ probe used was a 0.7-kb *Bgl*II-*Eco*RI fragment which contains the *Ke*⁻*Oz*⁺ gene of the locus (as shown in B). It is homologous to all C_λ genes on the locus. The λ IVS probe is a 2.7-kb *Bgl*II fragment derived from the region between the *Ke*⁻*Oz*⁻ gene (second from 5' end as shown in B) and the *Ke*⁻*Oz*⁺ gene (third from 5' end as shown in B). The latter probe is extensively homologous only to this region and to the region just 5' to the *Ke*⁻*Oz*⁻ gene. The designations *Ke*⁻*Oz*⁻ and *Ke*⁻*Oz*⁺ are the serotypes of the two proteins coded for by the two C_λ genes on the 8-kb fragment¹. (Note that the 5-kb band seen in lanes a-f appears reduced in lane b. This is an artefact and the consequence of having cut the nitrocellulose filter at the site where the 5-kb pseudogene fragment was located in lane b before hybridization. This individual does in fact have the pseudogene represented by this band.) B, Schematic of human λ constant region locus. The arrows indicate the *Eco*RI restriction sites on the locus. The location of 14- and 16-kb fragments, which are common to all genomic DNAs studied, are shown. The black boxes represent the C_λ genes which are present on the 14-, 16- and on 8-kb fragments. The presumed location of the 13-, 18- and 23-kb allelic fragments is indicated.

containing only an 18-kb genomic fragment gave rise to subpopulations containing 13- and 8-kb fragments, while the clone originally containing only a 13-kb genomic fragment gave rise to an 8-kb fragment-containing subpopulation. This region of the λ locus is obviously unstable when passaged through *E. coli*, apparently deleting a ~5- or 10-kb segment of DNA, and giving rise to *Eco*RI fragments identical in size to the polymorphic fragments seen in human populations.

To map the site of deletion in the phage DNA, heteroduplex analyses between intact and deleted phage clones were performed (data not shown). When the genomic 18-kb clone was heteroduplexed to the deleted 13-kb clone derived from it, an ~5.2-kb deletion bubble was consistently seen. Similarly, a 5.2-kb deletion bubble appeared when the original genomic 13-kb clone was heteroduplexed to its deleted 8-kb derivative. In both cases, the single deletion bubble could appear at multiple sites within the cloned fragment, an observation which could be explained by the presence within the clones of tandemly duplicated 5.2-kb regions of DNA. In addition, the deleted 8-kb derivatives from the 13- and 18-kb genomic clones showed precise homology to a phage clone containing a genomic 8-kb *Eco*RI fragment. These findings suggested that the deletions in the phage had created *Eco*RI fragments identical to those found in the polymorphic human λ alleles.

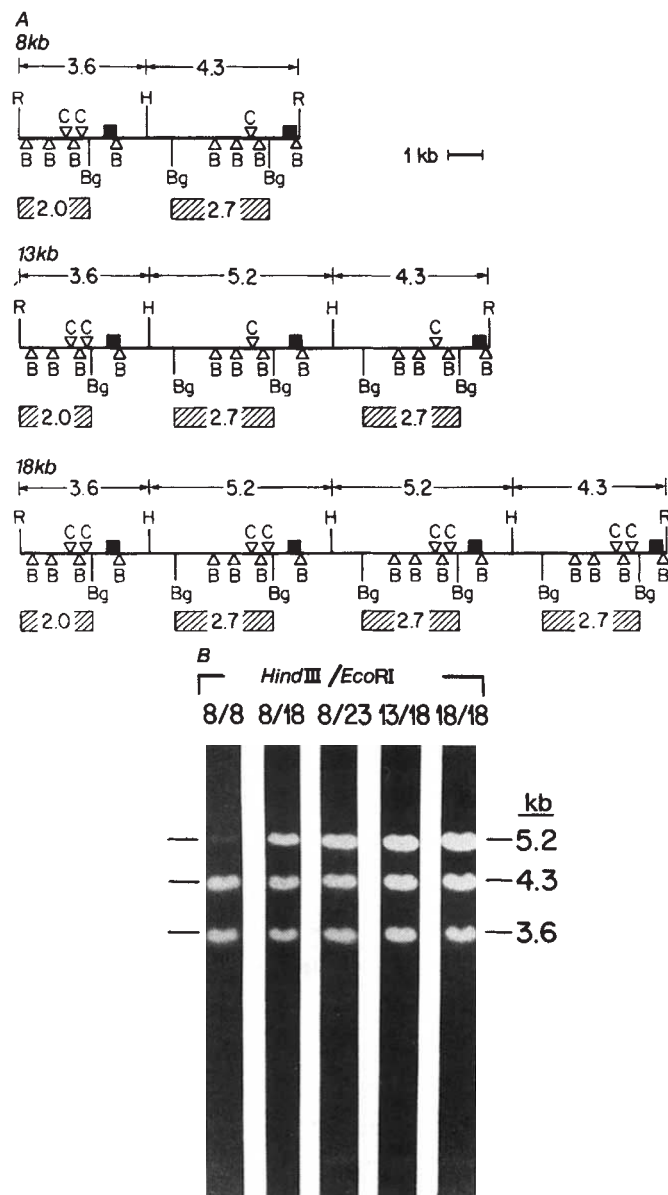


Fig. 2 A, Restriction maps of the 8-, 13- and 18-kb clones. R is *EcoRI*; B, *BamHI*; C, *CfoI*; H, *HindIII*; Bg, *BglII*. The sites of C_λ genes are indicated by black boxes. The sizes and location of $BglII$ -EcoRI fragments which hybridize to the 2.7-kb λ IVS probe are indicated by cross-hatched bars. The $HindIII$ -EcoRI fragments which hybridize to the λ IVS probe are indicated by arrows. B, Genomic DNA of various allotypes digested with $HindIII$ -EcoRI and hybridized to the λ IVS probe (method described in Fig. 1A legend). The 3.6-, 4.3- and 5.2-kb $HindIII$ -EcoRI fragments hybridize to the λ IVS probe.

When subcloned and grown in $recA^-$ bacteria, neither the 13- nor the 18-kb fragment underwent subsequent deletion. Restriction maps of the 13- and 18-kb subclones, as well as a more detailed map of the 8-kb clone, are shown in Fig. 2A. The 8-kb clone contains two C_λ genes, Ke^-Oz^- and Ke^-Oz^+ , that differ by only a few nucleotides. Each of these C_λ genes is surrounded by a virtually identical pattern of restriction sites which suggests that the DNA in this region is duplicated¹. The 13-kb clone contains exactly the same restriction site pattern plus a 5.2-kb inserted segment of DNA containing an additional C_λ gene (determined by probe homology) which is surrounded by an identical restriction site pattern. This clone seems to be a triplication of a basic ~5-kb repeat unit. The 18-kb *EcoRI* subclone has an identical restriction pattern except for the addition of another 5.2 kb of DNA containing a C_λ gene and the presence of two *CfoI* sites 5' to each of its C_λ genes. It thus

Table 1 Relative dosage of repeated segments in λ gene allotype and cloned DNA segments

a, Cloned DNA segments (ethidium bromide staining)		
Cloned segment	Calculated	Expected
	5.2-kb <i>HindIII</i> fragments	
13 kb	1.0	1.0
18 kb	1.8	2.0
	2.7-kb <i>BglII</i> fragments	
13 kb	1.9	2.0
18 kb	2.7	3.0
b, Genomic DNA (blot hybridization autoradiography)		
Allotype	Calculated	Expected
	5.2-kb <i>HindIII</i> fragments	
8/8	0	0
8/18	2.0	2.0
8/23	3.1	3.0
13/18	3.05	3.0
18/18	3.7	4.0

a, Densitometry tracings were made of the photographic negative of an ethidium-bromide stained gel on which the *HindIII*-EcoRI or *BglII*-EcoRI digested 13- and 18-kb subclones had been electrophoresed. In the case of the *HindIII*-EcoRI digestion, the number of 5.2-kb *HindIII* fragments in each clone was determined as follows:

$$A/B \times 4.3/5.2 \times 1 = N$$

where A is the integral of the 5.2-kb *HindIII* fragment densitometry tracing, B is the integral of the 4.3-kb *HindIII*-EcoRI fragment densitometry tracing and N is the calculated number of 5.2-kb *HindIII* fragments. The ratio 4.3/5.2 is a correction factor for the amount of ethidium bromide bound as a result of the molecular weight of each fragment. The number 1 is the number of 4.3-kb *HindIII*-EcoRI fragments known to be present in each clone. In a similar fashion, the number of 2.7-kb *BglII* fragments was calculated in each clone, based on the ratio of the 2.7-kb *BglII* fragment tracing to the tracing from the 2.0-kb *BglII*-EcoRI fragment known to be present in one copy in each clone. The number of fragments expected was determined from the molecular size of each clone and other restriction enzyme mapping and blot hybridization data. b, Densitometry tracings were made from the autoradiogram of a short exposure of the genomic blot shown in Fig. 2B. The number of 5.2-kb *HindIII* fragments in each allotype was determined as follows:

$$A/B \times 2 = N$$

where A is the integral of the 5.2-kb *HindIII* fragment densitometry tracing, B is the integral of the 4.3-kb *HindIII*-EcoRI fragment densitometry tracing and N is the calculated number of 5.2-kb *HindIII* fragments. The number 2 represents the number of 4.3-kb *HindIII*-EcoRI fragments in each allotype, given two genomic copies. The 'number expected' is based on the individual allotype as determined from Fig. 1. For instance, the individual with the 23-kb allele is 'expected' to carry five genes on the 23-kb *EcoRI* fragment given an insertion of three 5.2-kb repeat units of DNA. The data shown are consistent with this expectation.

apparently contains four copies of the basic unit. This was confirmed by determining the 'dosage' of each unit fragment from densitometry tracings of photographs of agarose gels on which restriction fragments from the two clones had been electrophoresed (see Table 1a, also Fig. 2A).

Based on nucleotide sequence and B-cell rearrangement data, the two λ constant genes found on the 8-kb *EcoRI* fragment are expected to be active genes coding for known λ proteins (Ke^-Oz^- , Ke^-Oz^+)¹. We do not know if the extra genes on the 13-kb and 18-kb alleles are active, but heteroduplex and restriction enzyme mapping analyses indicate that extensive homology to active genes has been retained.

Because cloning and growing the 13- and 18-kb genomic *EcoRI* fragments in λ CH4A could have resulted in alterations of their DNA, and because the 23-kb allele had not been cloned, restriction enzyme site patterns predicted from the cloned fragments were examined in genomic DNA. This was done by digesting various allotype genomic DNAs with *HindIII*-EcoRI, using the blot hybridization technique with a λ IVS probe (see Fig. 1) to detect and quantify homologous fragments. As can

be seen by comparing the restriction site maps of the cloned fragments shown in Fig. 2A with the blot hybridization analysis of doubly digested genomic DNA shown in Fig. 2B, only the predicted fragments hybridize extensively to the λ IVS probe. In addition, densitometry tracings of short-exposure autoradiograms of these blots were performed, and the number of 5.2-kb *Hind*III fragments calculated from the tracings was consistent with predictions based on the allotype of the particular DNA examined (Table 1b). Longer exposures of these autoradiograms are shown in Fig. 2B.

The occurrence of a 5.2-kb repeat unit within the λ locus immediately suggests a simple mechanism for generating the expanded and contracted polymorphic forms of the locus. Extensive homology between large repeats presents a convenient and homologous target for unequal crossing-over during meiosis. Figure 3 is a diagrammatic representation of the mechanism in which an unequal cross-over event occurs between two 8-kb fragments resulting in 13- and 3-kb *Eco*RI fragments. The 13-, 18- and 23-kb fragments observed could be explained by such a mechanism. An occasional consequence of unequal crossing would be a 3-kb allele. However, among the ~200 alleles examined, we have not found a 3-kb *Eco*RI fragment with homology to this λ IVS probe. Perhaps there are selective mechanisms that favour alleles with more than one gene copy in this segment. (In fact, in a few cases, we have noted a 3-kb *Eco*RI fragment with homology to the C_λ probe and to an intervening sequence probe derived from *mclg*, the 5'-most λ gene of the locus¹. This fragment is unrelated to the 8-, 13-, 18- and 23-kb polymorphic fragments which are located in the region containing the next two λ genes of the locus, *Ke-Oz*⁻ and *Ke-Oz*⁺. It is possible that this uncharacterized 3-kb fragment represents an additional allele of the 5' most λ gene, *mclg*.)

In the immunoglobulin system the number of gene copies can be expanded at little cost and possibly some advantage to the organism. This is in contrast to other gene systems—the globins, for example—in which extra copies of globin genes would be expected to unbalance their regulated expression. As immunoglobulin genes require a site-specific recombination event for their activation⁸⁻¹⁰ and genes that remain in the germ-line configuration cannot produce an active gene product,

extra copies of the J/C λ unit²⁻⁵ would simply contribute to the organism's potential for generating diversity without disrupting the delicate stoichiometry between heavy and light chains that is required for the formation of an active antibody. Such a 'forgiving' system should give rise to analogous polymorphisms in any genetic system that depends on combinatorial mechanisms to form active genes.

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A minimum of four human class II α -chain genes are encoded in the HLA region of chromosome 6

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The major histocompatibility complex (MHC) in man, also called the HLA region, is located on the short arm of chromosome 6 and encodes antigens involved in immunological processes¹. The class II HLA antigens consist of two noncovalently associated polypeptide chains, one of molecular weight 34,000 (α) and the other of molecular weight 29,000 (β)². The extensive polymorphism of the β chain(s) has allowed the genetic mapping of the corresponding β gene(s) to the HLA-DR region³⁻⁵. cDNA clones for the HLA-DR α chain have been used to map the non-polymorphic DR α -chain gene to chromosome 6 using mouse-human somatic cell hybrids⁶. Similarly, the DR α -chain gene⁷ has been mapped to the short arm of chromosome 6 centromeric to the HLA-A, -B and -C loci by *in situ* hybridization experiments⁸. We isolated a cDNA clone that is related to the DR α chain and encodes the class II antigen DC α chain⁹. We describe here how this DC α clone was used to find two or three additional α -chain genes by cross-hybridization and how HLA-antigen loss mutants of a human lymphoblastoid cell line (LCL) were used to ascertain that these additional class II antigen α -chain genes are also located in the HLA region.

First, we produced mutants that no longer expressed HLA-DR antigens as a result of the introduction of two γ -ray-induced mutations. Some of the DR-null mutants still expressed a DC antigen (that is, were DR-null, DC⁺) while others no longer expressed DC (that is, were DR-null, DC-null). We then determined that a radioactive probe for the DC α gene reassociated with a specific restriction fragment of DNA from DR-null, DC⁺ cells but that the specific fragment was absent in DNA from

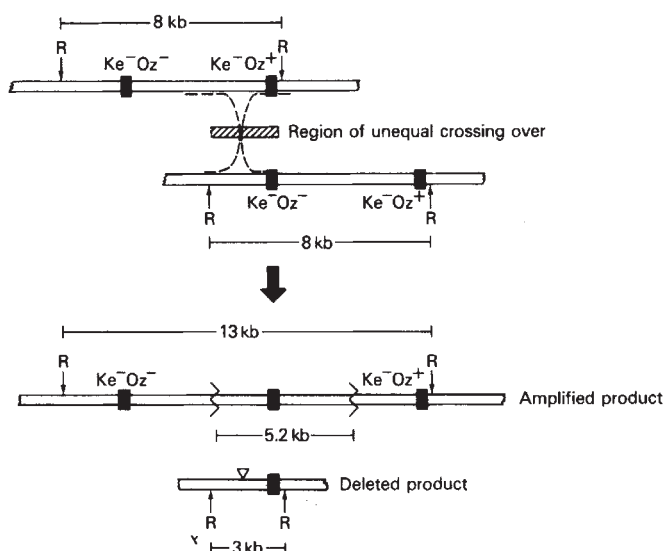


Fig. 3 Proposed mechanism for generation of λ polymorphisms. Cross-over event between two 8-kb *Eco*RI (R) allelic fragments is shown. The region of possible unequal crossing-over was determined from mapping data in which extensive homology was demonstrated in the region extending from just to the right of the 5' *Eco*RI site to just to the left of the 3' *Eco*RI site. The two products, one 13 and one 3 kb, which would be generated, are depicted below the black arrow. The 5.2-kb insertion in the 13-kb fragment is indicated. The deletion site in the 3-kb fragment is designated by an open triangle.

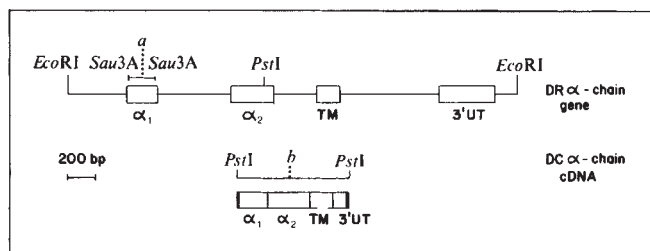


Fig. 1 DNA probes used in this study. A schematic diagram of the two clones from which the probes are derived is presented. α_1 , α_2 , First and second extracellular domains; TM, transmembrane region; 3'UT, 3'-untranslated region of the mRNA. *a*, A 230-bp *Sau3A*-*Sau3A* restriction fragment derived from the DR α -chain gene⁷. pUR5(3.2RI) DNA was digested with *EcoRI* and *PstI* and the 1.4-kb *EcoRI*-*PstI* fragment containing exon α_1 and part of exon α_2 was isolated by agarose gel electrophoresis using the glass bead procedure²⁷, the fragment was then redigested with *Sau3A* and the 230-bp fragment corresponding to the α_1 exon was isolated from a 5% polyacrylamide gel by electroelution, and recovered by DEAE-cellulose chromatography. *b*, The *PstI* insert (800 bp) of pDCH1, which contains the sequence for the mature polypeptide chain except the first 17 amino acids⁹, was excised with *PstI* from pDCH1 and isolated by agarose gel electrophoresis using the glass bead procedure²⁷.

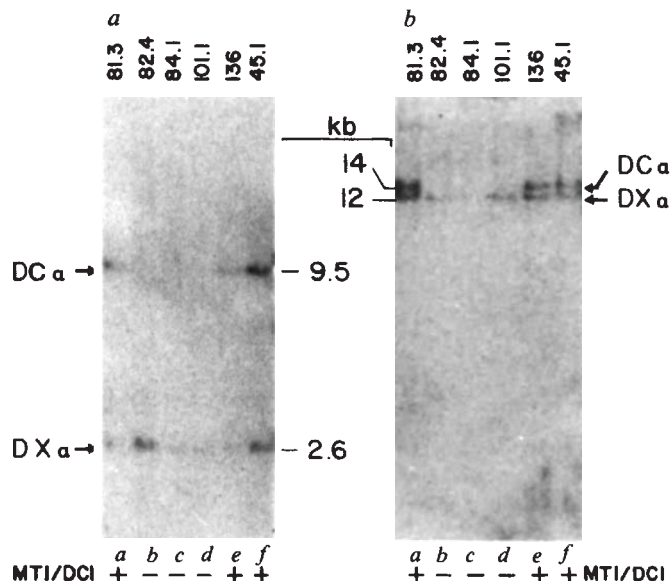


Fig. 2 DNA filter hybridization analysis of deletion mutants with the pDCH1 DC α -chain probe. Lanes *a*-*e*, DR-null mutants derived from mutant 45.1; lane *f*, mutant 45.1. The MT1/DC1 phenotype of each mutant is indicated below each lane. High-molecular weight DNA was isolated from the different cell lines as described previously²⁸. After complete digestion with *HindIII* (*A*) or *BamHI* (*B*), 15 μ g of each DNA were electrophoresed in a 0.8% agarose gel. DNA was depurinated by immersing the gel in 0.25 M HCl for 30 min and then denatured in 0.5 M NaOH, 1 M NaCl for 30 min. The gel was then neutralized in 0.5 M Tris-HCl, pH 8.0, 3 M NaCl for 30 min and the DNA was transferred to a nitrocellulose filter (Schleicher and Schuell) as described previously²⁹. The filter was baked for 2 h at 80 °C under vacuum, then prehybridized for 4 h at 68 °C in 2 $\times$ SET (SET = 0.15 M NaCl, 1 mM EDTA, 50 mM Tris, pH 7.8), 5 $\times$ Denhardt's solution, 50 mM NaH₂PO₄, pH 6.5, 0.1% SDS and 0.1 mg ml⁻¹ sonicated, denatured salmon sperm DNA. Hybridization was performed for 16 h at 68 °C in the same conditions as above with the addition of 10% dextran sulphate and 10⁷ c.p.m. of ³²P-labelled pDCH1 insert (Fig. 1*b*) labelled by nick-translation³⁰ at a specific activity >10⁸ c.p.m. per μ g. The filter was washed twice for 1 h at 68 °C in each of the following solutions: 2 $\times$ SET, 0.2% SDS; 1 $\times$ SET, 0.2% SDS; and 0.5 $\times$ SET, 0.2% SDS, then dried and exposed to a Kodak XAR-5 X ray film for 4 days with an intensifying screen (Dupont). Cross-hybridization between DC α and DR α is observed only above 2 $\times$ SET. Sizes of hybridizing DNA fragments were determined by comparison with λ *HindIII* restriction fragments.

technique with a radioactive DC α probe (Fig. 2). After washing the filters in conditions that do not allow detection of the DR α -chain gene, two bands were detected in both cases. The only obvious difference seen between the cell lines is the absence of the largest band in the three DR-null, MT/DC-null, SB⁺ mutants. We conclude from this experiment that the loss of expression of the MT1/DC1 phenotype is correlated with loss of the DC α -chain gene. A similar result was obtained with a third restriction enzyme, *EcoRI*. Note that the other DNA fragment, designated DX α , is not deleted or modified in any of the mutants, suggesting that it corresponds to another closely related gene or pseudogene (see below). All of these DR-null mutants still expressed the B5 and A2 alleles on the telomeric side of DR¹³ and the SB2 allele on the centromeric side^{13,15}. Therefore, the deletion of the DC α -chain gene that occurs when expression of the *cis*-linked DR1 and MT1/DC1 specificities are simultaneously lost maps the DC α -chain gene to the DR region on chromosome 6.

Because the first experiment (Fig. 2) showed clearly that the human genome contains at least one other DC-like α -chain gene, we further analysed the same cell lines with the DC α probe in more sensitive conditions for detection of small DNA fragments and cross-hybridizing sequences (Fig. 3). Although

DR-null, DC-null cells. In contrast, a probe for the DR α gene revealed its presence in all of the DR-null mutants we studied. This suggests that the DR-null phenotype resulted from the loss of part of the DR α gene or from the loss of the DR β gene(s). The DC α probe detects at least two more α -chain genes, which are absent in mutants that have lost expression of all class II antigens (SB, DC and DR). The HLA region therefore appears to contain at least four α -chain genes.

We have reported previously the isolation and characterization of the HLA-DR α -chain gene⁷, and the use of DNA probes derived from this gene to isolate a cDNA clone encoding the DC α chain⁹. This was possible because of the nucleotide sequence homology between the exons coding for the second, immunoglobulin-like, extracellular domain (α_2) and the transmembrane regions of both chains. To probe for the DR α -chain gene in the DR-null mutants, we have used as a specific probe the 230-base pair (bp) *Sau3A*-*Sau3A* DNA fragment of the DR α gene that corresponds to the first extracellular domain (α_1) where the homology with the DC α -chain sequence is low (Fig. 1*a*). The complete *PstI* insert (800 bp) from the DC α cDNA clone pDCH1 has been used to probe for the DC α -chain gene and related sequences (Fig. 1*b*).

The cell mutants we used were derived from LCL 721, which has the haplotypes: (1) GL01,HLA-[SB2,MT1,DR1,B5,A2] and (2) GL02,HLA-[SB4,MT2,DR3,B8,A1]. MT1 has been shown to be identical to DC1, MB1 and LB12 (ref. 10). Treatment of LCL 721 with γ rays (300 rad) followed by selection with an anti-B8 serum and complement resulted in isolation of diverse mutants, among which was 45.1, which expressed haplotype (1) above, no longer expressed haplotype (2) and had a prominent microscopically visible deletion on the short arm of one chromosome 6 (refs 11, 12). Mutant 45.1 was irradiated a second time and selection with complement and the monoclonal antibody L243 (Becton-Dickinson), which is directed against a monomorphic DR determinant, permitted the isolation of DR-null mutants that neither expressed the DR1 and DR3 antigens nor bound L243, as determined by cytotoxicity tests and enzyme-linked immunosorbent assay^{13,14}. Some of the DR-null mutants expressed MT1, but others were DR-null, MT-null^{13,14}. Furthermore these mutants were SB⁺, but mutants which were SB-null, DR-null and MT-null were also obtained (refs 13, 15 and R.DeM., unpublished).

DNA from mutant 45.1 and five DR-null mutants derived from it, three of which had also lost expression of the MT1/DC1 determinant, but all of which were SB⁺, was digested with *HindIII* or *BamHI* and analysed by the filter hybridization

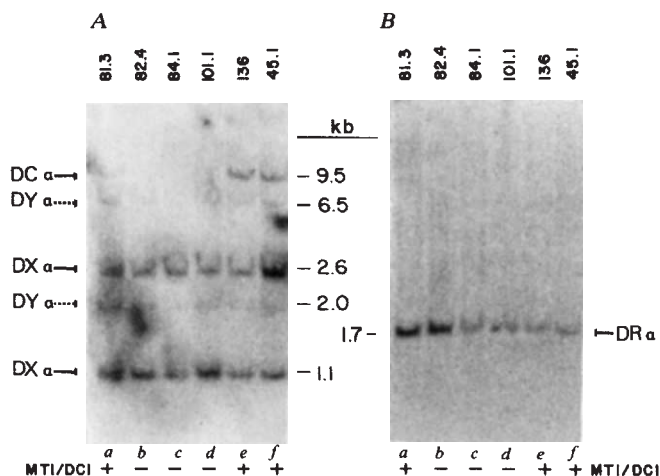


Fig. 3 Detection of DNA sequences distantly related to the DC α -chain gene. **A**, The experiment was performed with *Hind*III-digested DNA as described in Fig. 2A legend with the following modification: in order to obtain a better transfer of small DNA fragments, the depurination and denaturation steps were reduced to 20 min each and Genescreen (NEN) was used as the nitrocellulose filter. Hybridization with the pDCH1 insert (Fig. 1b) and washing of the filter were performed as described in Fig. 2 legend. After autoradiography, the first probe was eluted by incubating the filter for 1 h at 70°C in 5 mM Tris-HCl pH 8.0, 0.2 mM EDTA, 1 mM NaHPO₄, pH 6.5 and 0.1 $\times$ Denhardt's solution. **B**, A second hybridization was performed with the filter used in **A**, but using the DR α_1 exon probe (Fig. 1a) and finally the filter was washed twice for 1 h in 1 $\times$ SET at 68°C. Sizes of hybridizing DNA fragments were determined by comparison with λ *Hind*III restriction fragments. Lanes a-e, DR-null mutants derived from 45.1; lane f, 45.1. The MT1/DC1 phenotype of each cell line is indicated below each lane. DX α and DY α correspond to the strongly and weakly cross-hybridizing fragments, respectively.

the final conditions of washing were similar to those of the previous experiment (Fig. 2A), in addition to the two *Hind*III fragments already detected, three more DNA fragments hybridized to the DC α probe (Fig. 3A). None of these fragments is deleted in the DR-null mutants, regardless of their MT/DC phenotype. That the two prominent *Hind*III fragments designated DX α (2.6 kb and 1.1 kb) correspond to a single gene or pseudogene with a close relationship to the DC α -chain gene is supported by the fact that we have found them to be linked in a genomic clone isolated with the DC α probe. In addition, the 2.6-kb *Hind*III DX α fragment has been detected in five HLA-DR homozygous typing cells¹⁶.

Although we did not expect to find cross-hybridization between the DC α -chain cDNA sequence and the DR α -chain gene in the conditions used to wash the filter, it was not formally excluded that one of the faint hybridizing bands might correspond to the DR α -chain gene. After washing off the first probe, the same filter was hybridized with the DR α probe (Fig. 3B). A single DNA fragment corresponding to the 5' end of the unique DR α -chain gene^{6,7} was detected with this DR α_1 probe in all cell lines, and its size does not correspond to any of the DNA fragments detected with the DC α probe. Moreover, its size is the same in all cell lines, indicating that at least part of the DR α -chain gene is probably retained. This observation suggests that the DR-null phenotype resulted from the loss of DR β -chain genes that are not close to the DR α -chain gene and are most probably located between the DC α - and DR α -chain genes. Alternatively, the DR-null phenotype might have resulted from loss of part of the DR α -chain gene that does not hybridize with the 230-bp probe we used. The 2.0-kb and 6.5-kb DY α fragments hybridizing weakly with the DC α probe shown in Fig. 3A therefore are not DR α and represent one or two more α -chain gene(s).

Deletion mutants have been used to confirm that another recently discovered class II antigen, SB¹⁷⁻¹⁹, is encoded in a

region distinct from DR and MT^{13-15,19}. Recently, it has been shown that the SB antigen has a structure typical of class II antigens²⁰, and partial amino-terminal sequencing of the SB α chain has suggested that it is more closely related to the DR α chain than to the DC α chain²⁰. SB2 was expressed in all the DR-null mutants described above, whether MT/DC-null or not^{13,15}. DNA from two additional mutants (174 and 180) which lack expression of SB as well as DR and MT were analysed with the DR α and DC α probes. Both probes failed to detect any DNA fragment in these mutants, that is, they were DR α -, DC α -, DX α - and DY α -negative. The simultaneous deletion of DX α and DY α along with DR α and DC α in these mutants indicates that these genes are also localized in the HLA region of chromosome 6. Moreover, at least one of the additional fragments detected with the DC α probe might contain the SB α -chain gene. This is the first documented evidence for the occurrence of a minimum of four α -chain genes in the haploid human genome. They all seem to be located on the short arm of chromosome 6 in the HLA complex. This finding is in marked contrast with the situation found in the mouse I region where only two α -chain genes, A α and E α , have been described^{16,21,22}. One possible explanation is that duplication events have taken place in the HLA-DR region after the mouse/human speciation, one of which has occurred recently, giving rise to the closely related DC α and DX α genes. As the large deletions present in the DR-null MT/DC-null mutants do not encompass α -chain genes other than DC α , it seems likely that the α -chain genes are not clustered but rather widely dispersed in the HLA region. Additional deletion mutants may assist in ordering these α -chain genes as well as the class II β -chain genes for which probes are available (refs 23, 24, and M. Roux-Dosseto *et al.*, unpublished). The same principle is being successfully used to order the HLA class I genes of LCL 721 (refs 25, 26).

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Note added in proof: A cDNA clone corresponding to the SB α chain has been isolated and was used in recent experiments to show that the SB α -chain gene does not correspond to any of the α -chain genes described in this report (C.A. *et al.*, unpublished). The minimum number of α -chain genes is, therefore, either five or six (one DR α , three or four DC α -related and one SB α).

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Myositis autoantibody inhibits histidyl-tRNA synthetase: a model for autoimmunity

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In autoimmune disorders such as rheumatoid arthritis and systemic lupus erythematosus (SLE), autoantibodies are generated against a variety of macromolecules. Myositis is a human autoimmune disease characterized by weakness and wasting of muscle¹. In American studies^{2,3}, antibodies directed against soluble cellular constituents were detected by immunodiffusion in about 60% of cases; the commonest of these, found in 25% of patients, was antibody to the Jo-1 antigen. An antibody system referred to as PL-1 was recognized at a similar frequency in a series of patients studied at Hammersmith Hospital, London⁴. We show here that this system is identical with the Jo-1 system and demonstrate that the antigen is a polypeptide of molecular weight (M_r) 50,000. The protein is immunoprecipitated with tRNA^{His} and appears to be histidyl-tRNA synthetase. The identity of the Jo-1 antigen, the first of the RNA-associated antigens familiar in autoimmune disease^{5,6} to be characterized as a specific enzyme, suggests a model for virus involvement in autoantibody generation.

Serum samples from patients with polymyositis, dermatomyositis and other rheumatic conditions were screened for precipitating antibodies to soluble cellular antigens by counter-immunoelectrophoresis⁴. IgG preparations from seven sera containing the PL-1 antibody were used in immunoprecipitations of HeLa cell extracts labelled with either ³⁵S-methionine or ³²P-phosphate⁷. A protein of M_r 50,000 (Fig. 1) and RNA of tRNA size (Fig. 2a) were precipitated by all PL-1-positive sera and also by two anti-Jo-1 reference sera. These species were not recognized by normal sera, nor by sera containing other autoantibodies, including those from PL-1-negative myositis cases. As expected from the serological tests, two sera contained a further antibody (anti-(U1)RNP and anti-La in separate cases) and accordingly precipitated additional characteristic protein and RNA species. As subsequent studies have confirmed the immunological identity of PL-1 and Jo-1, we adopt the original cognomen for the remainder of this report.

The co-precipitation of protein and RNA species by anti-Jo-1 antibody suggested that both were components of a complex. To discover which species contained the antigenic site we carried out immunoprecipitations with ³⁵S-labelled extracts digested with RNase. As shown in Fig. 1, the 50,000 M_r protein remained precipitable after digestion, in contrast to the (U1)RNP-specific polypeptides. Conversely, the RNA was not efficiently precipitated from ³²P-labelled extracts deproteinized by phenol (Fig. 2b). These results demonstrate that the antigenic determinants reside in the protein moiety. Furthermore, when a ³²P-labelled extract was digested with RNase, no RNA was detectable in the precipitate, indicating that the RNA is not protected within the ribonucleoprotein complex. However, the immunoprecipitate did contain a 50,000 M_r polypeptide weakly labelled with ³²P (not shown), suggesting that the antigen is a phosphoprotein. The antigenic complex was detected in the

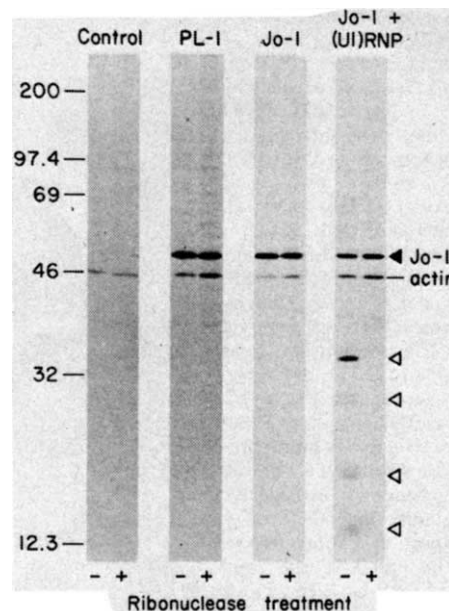


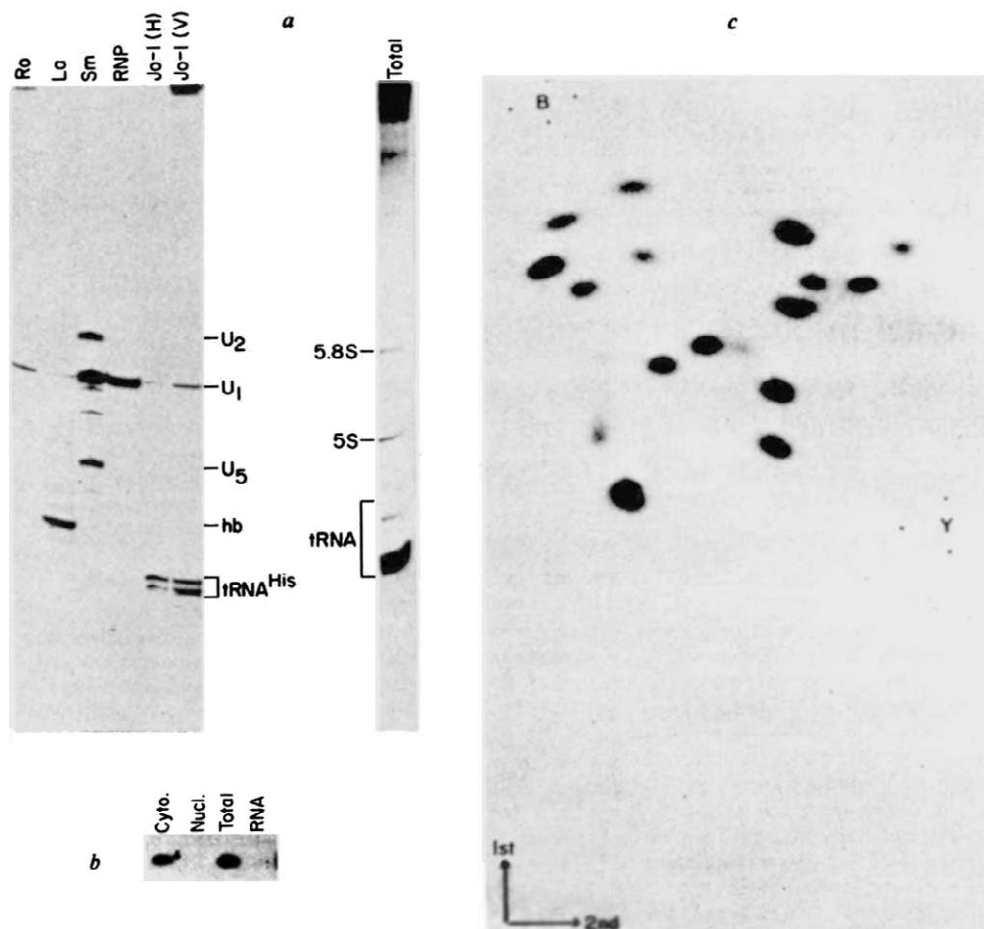
Fig. 1 Protein immunoprecipitated by anti-Jo-1 autoantibodies. ³⁵S-methionine-labelled HeLa cell extracts were prepared and used in *Staphylococcus aureus* protein A-mediated immunoprecipitations essentially as described by Francoeur and Mathews⁷. Polypeptides were detected by SDS-polyacrylamide gel electrophoresis and fluorography. As indicated below the autoradiogram, a portion of the extract was incubated with 0.5 mg ml⁻¹ pancreatic RNase for 5 min at 37 °C before addition of antibody. IgG fractions came from normal serum (Control) and from three patients with anti-Jo-1 antibodies: patient H, the PL-1 (now Jo-1) prototype; Jo-1 reference serum supplied by M. Reichlin; and patient M with (U1) RNP as well as PL-1 (Jo-1) antibodies. The Jo-1- and RNP-specific polypeptides are indicated (closed and open arrowheads, respectively), and the positions of molecular weight standards and nonspecifically precipitated actin are marked.

cytoplasmic but not the nuclear fraction of ³²P-labelled HeLa cells (Fig. 2b), and indirect immunofluorescence of HeLa cell monolayers revealed weak cytoplasmic staining (not shown). These observations support a cytoplasmic⁸ rather than a nuclear² localization for the antigen.

The minor nucleotide content (data not shown) and probable cytoplasmic localization of the RNA, taken together with its size, reinforced the hypothesis that the RNA component of the antigen is a transfer RNA. High resolution polyacrylamide gel electrophoresis of the RNA immunoprecipitated by each anti-Jo-1 serum revealed two major bands (Fig. 2a). The ratio of these bands varied somewhat between experiments, and a third slightly faster band was sometimes visible. The complexity of the RNA was also assessed by two-dimensional fractionation of RNase T₁ digested material. The resultant fingerprint (Fig. 2c) was consistent with the presence of a unique tRNA species, possibly containing minor variations in a single oligonucleotide. Essentially identical fingerprints were obtained from RNA precipitated by two of our anti-Jo-1 sera, and by Rosa *et al.*⁸ with anti-Jo-1 reference serum. Using direct sequence analysis, these workers have shown that this RNA is a tRNA containing the anticodon for histidine, closely resembling histidine-accepting tRNA species from *Drosophila*⁹ and sheep¹⁰.

These observations led us to postulate that the Jo-1 antigen is histidyl-tRNA synthetase. To test this idea we added increasing amounts of anti-Jo-1 IgG to an extract of HeLa cells which contained the activities required for joining amino acids to their cognate tRNAs. Figure 3a and b show that the charging of tRNA with ³H-histidine was rapidly and efficiently blocked by the antibody. The inhibition was specific for histidine, not being seen with any of the other 19 amino acids. Furthermore, the inhibition of histidine charging was specific for anti-Jo-1, as it was seen with all nine anti-Jo-1 sera but with none of the control sera (2 normal sera and 10 sera with various different

Fig. 2 Analysis of Jo-1-specific RNA. *a*, High resolution gel of RNA immunoprecipitated from 32 P-labelled HeLa cell extracts by IgGs from Jo-1 sera (patients H and V). Other Jo-1 sera yielded similar RNA bands in the tRNA region, with variable amounts of high molecular weight material. Markers include RNAs precipitated by the standard SLE autoantibodies Ro, La, Sm and (U1)RNP, as well as the RNA isolated by phenol from the cell extract (Total). Electrophoresis was through a urea/10% polyacrylamide thin gel run in half-strength Tris/borate/EDTA buffer. Small amounts of (U1)RNA were precipitated nonspecifically in all cases. Visualization of Ro-specific RNAs requires longer autoradiographic exposure. *b*, Immunoprecipitation by anti-Jo-1 IgG from fractionated 32 P-labelled HeLa cell extract. The immunoprecipitates from cytoplasmic (Cyto.) and nuclear (Nucl.) fractions, whole cell extract (Total) and deproteinized RNA isolated from the whole cell extract (RNA), were resolved in an SDS/20% polyacrylamide gel. *c*, Fingerprint of RNA precipitated by anti-Jo-1 IgG (patient H). After gel electrophoresis, tRNA-sized material was eluted, digested with RNase T₁, and 'fingerprinted' by cellulose acetate electrophoresis (first dimension) followed by DEAE-cellulose homochromatography (second dimension). Essentially identical fingerprints were obtained without gel purification of the RNA, and from RNA precipitated by another anti-Jo-1 IgG (patient A). This and other methods were described by Francoeur and Mathews⁷.



autoantibodies, including three associated with myositis). Examples of these controls are shown in Fig. 3. A further control demonstrated that anti-Jo-1 IgG had no effect on the stability of histidyl-tRNA (data not shown). In a second type of experiment we removed the Jo-1 antigen from the aminoacyl-tRNA synthetase preparation by treatment with anti-Jo-1 IgG immobilized on protein A-Sepharose. The depleted extract was unable to catalyse the charging of added tRNA with histidine although its activity with other amino acids was undiminished (Fig. 3c). Treatment with immobilized normal IgG or with anti-La IgG (not shown) had no effect. These data indicate that anti-Jo-1 IgG and not a contaminant is responsible for the inhibition of aminoacylation.

We conclude that the 50,000 *M_r* Jo-1 antigen is a subunit of histidyl-tRNA synthetase. This enzyme has been purified from rabbit reticulocytes as a dimer of similar or identical subunits of *M_r* ~64,000 (ref. 11): its native molecular weight was estimated at 122,000. On the other hand, the *M_r* of the Jo-1 antigen partially purified from calf thymus appeared to be about 150,000 (ref. 2). These disparities in molecular weight are probably unimportant, and presumably represent species differences or imprecisions in molecular weight estimation. We have also considered the possibility that the 50,000 *M_r* protein is an ancillary factor required by the enzyme, but high molecular weight cofactors are not generally required by aminoacyl-tRNA synthetases, and the purified reticulocyte enzyme was active in the absence of any such factor. A second alternative would suppose the 50,000 *M_r* protein to be unrelated and precipitated by a second antibody. This hypothesis is rendered unlikely by the complete concordance of enzyme inhibition, tRNA^{His} precipitation and 50,000 *M_r* protein precipitation among the nine sera tested. Ultimately, these alternatives can be ruled out only by purification of the enzyme, but they already appear

implausible.

Muscle destruction in myositis is probably caused by cytotoxic lymphocytes^{12,13}. On the other hand, the concomitant inflammation, in which immunoglobulin and complement are deposited in small blood vessels¹⁴, may be the result of a humoral immune disorder. The possibility that autoantibodies penetrate living cells and exert a direct cytotoxic effect appears remote but is readily testable with anti-Jo-1 antibody.

Antibodies directed against tRNAs or tRNA-associated proteins seem to be a hallmark of myositis^{4,6,8}, in contrast to SLE in which antibodies to DNA, histones and nuclear ribonucleoprotein particles abound⁴⁻⁶. This difference may simply reflect the exceptionally high ratio of cytoplasm to nuclei in muscle tissue, coupled with the cycle of damage and regeneration occurring in myositis. However, this explanation would not account for the specific immune response to the Jo-1 antigen unless histidyl-tRNA synthetase is particularly immunogenic or persistent. An attractive alternative hypothesis postulates that the anti-Jo-1 response results from the binding of histidyl-tRNA synthetase to a viral RNA. Nucleic acid is known to enhance the immunogenicity of histones¹⁵, and a viral aetiology for myositis has been proposed¹⁶. In particular, coxsackievirus B, a picornavirus capable of infecting muscle¹⁷, has been implicated¹⁸. It has been reported that the RNA genomes of two related viruses can be charged at internal sites with amino acids, encephalomyocarditis virus RNA with serine¹⁹ and mengovirus RNA with histidine²⁰. If myositis were to follow infection with a myotropic virus capable of carrying a histidine residue attached to its RNA, the consequent cell damage and release of viral RNA complexed with histidyl-tRNA synthetase might then overcome immunological tolerance, provoking the generation of antibody to the charging enzyme itself. Noting that the cellular protein La forms complexes with viral RNAs in

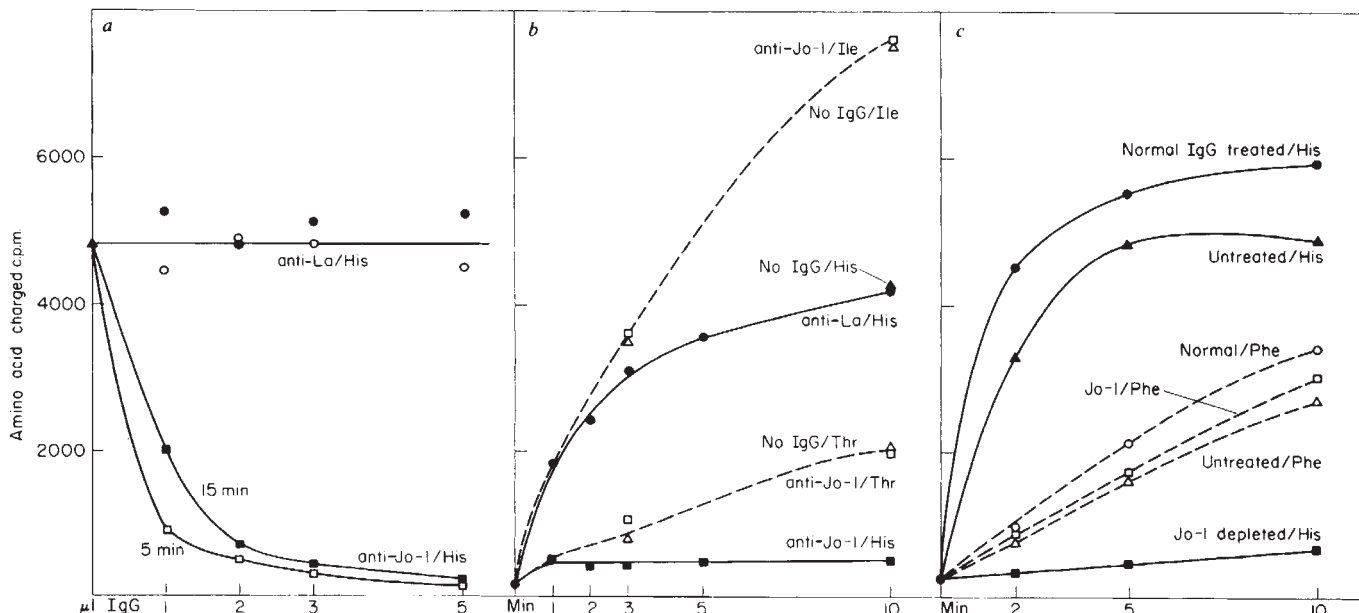


Fig. 3 Inhibition of histidyl-tRNA synthesis by anti-Jo-1 and anti-La antibodies added in increasing amounts. Incubations contained ^3H -histidine and anti-Jo-1 IgG (patient H; squares) or anti-La IgG (circles). Reactions were terminated after 5 min (open symbols) and 15 min (closed symbols). **b**, Kinetics of aminoacylation in the presence of antibodies. Incubations contained anti-Jo-1 IgG (patient H; squares), anti-La IgG (circles) or equivalent buffer (triangles), and one of the three ^3H -labelled amino acids (histidine: closed symbols, solid lines; isoleucine or threonine: open symbols, broken lines). **c**, Aminoacylation in Jo-1-depleted extracts. To remove antigens, HeLa cell extracts were treated with protein A-Sepharose beads carrying IgG from an anti-Jo-1 serum (patient H; squares) or normal serum (circles), essentially as described previously⁷. The treated extracts were diluted approximately twofold in the process, so were assayed at 10 μl per reaction instead of 5 μl as used for untreated extract (triangles). Reactions contained ^3H -histidine (closed symbols, solid lines) or ^3H -phenylalanine (open symbols, broken lines). Aminoacylation reactions typically contained, in a volume of 25 μl : 5 μl of a dialysed 100,000g supernatant from HeLa cell cytoplasm prepared essentially as described by Mathews and Korner²³; 5 μl IgG at $\sim 1\text{ mg ml}^{-1}$; 90 μg calf liver tRNA (Boehringer); 3 μCi radioactive amino acid plus 16 μM of the same amino acid in unlabelled form; 5 mM ATP, 0.5 mM GTP and 0.5 mM CTP; 12 mM Tris-HCl, pH 7.4; 10 mM MgCl_2 , 30 mM KCl, 30 mM NaCl, 2 mM Na phosphate, 0.1 mM EDTA and 0.6 mM dithiothreitol. Incubation was at 37°C and 5- μl samples were removed at various times for determination of the amount of amino acid bound to tRNA. The aliquot was pipetted onto a filter-paper square which was rinsed several times in ice-cold 10% trichloroacetic acid (TCA) solution containing the unlabelled amino acid at 1 g l^{-1} . After washing with ethanol and drying, the paper squares were assayed for radioactivity in a liquid scintillation counter. Incorporation of histidine into TCA-insoluble form was dependent on added tRNA. The identity of this material with histidyl-tRNA was confirmed by its sensitivity to boiling TCA, to digestion by RNase and to incubation with 0.5 M Tris, pH 9.2. It remained in the aqueous phase during phenol extraction.

adenovirus infected and Epstein-Barr virus-transformed cells^{21,22}, a similar explanation was proposed for the origin of anti-La antibodies in SLE. The combination of a cellular protein with altered or foreign nucleic acid may be a general feature of the autoimmune response.

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A component of *Drosophila* RNA polymerase I promoter lies within the rRNA transcription unit

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Genes transcribed by RNA polymerase II have been characterized as having several control regions upstream of the site at which transcription is initiated, and the 'CAAT' and 'TAATA' regulatory sequences are conserved in content and location in diverse eukaryotes¹⁻³. The transcription of 5S RNA and tRNA by polymerase III is regulated by promoters that are actually within the genes⁴; these are also conserved in diverse eukaryotes⁵. In addition, sequences upstream of tRNA genes can affect RNA synthesis^{6,7}. Recently, it has been determined that DNA sequences influencing the expression of the 18S and 28S ribosomal RNA genes (rDNA) lie upstream of the site of transcription initiation⁸⁻¹². We have shown⁹ that a major component of the promoter of *Drosophila* rRNA polymerase I activity involves the region -43 to -27, where initiation is at +1. Here we present evidence that another major component of the polymerase I promoter lies within the first four nucleotides of the external transcribed spacer (ETS). *Drosophila* polymerase I control signals, therefore, lie both upstream of and within the rRNA transcription unit.

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To localize sequences that are involved in the promotion of polymerase I transcription, we used the exonuclease *BAL31* to prepare a series of recombinant plasmids containing decreasing amounts of either nontranscribed spacer (NTS)⁹ or ETS sequences (Fig. 1). These plasmids were evaluated for their ability to act as templates for run-off transcription in an S100 extract of *Drosophila melanogaster* Kc cells^{8,9,13}. This *in vitro* system has been shown to direct the faithful and accurate transcription of exogenous *D. melanogaster* rDNA by RNA polymerase I¹³.

The plasmid pDmr275c2 (ref. 8), which was subjected to *BAL31* digestion, contains a segment of *D. melanogaster* rDNA extending from an *AluI* cleavage site at -305 to a *HaeIII* site at about +680 (Fig. 1). The rDNA segment was inserted between the *HindIII* and *BamHI* sites in pBR322, with the ETS *HaeIII* site fused to the vector *BamHI* site so that the direction of transcription was towards the *BamHI* site. pDmr275c2 was linearized with *BamHI*, treated with *BAL31* to various extents of digestion, then *EcoRI* linkers were added to the deletion termini⁹. There is a unique *ClaI* site in the vector, 6 base pairs (bp) upstream of nucleotide -305 of the NTS; *ClaI/EcoRI* digestion and preparative gel electrophoresis were used to separate the ETS-deleted rDNA inserts from the vector and to reduce oligomeric linkers to monomer. The shortened *Drosophila* rDNA segments were then ligated to the *EcoRI* and *ClaI* sites of pBR322 (this results in the orientation of the inserts with respect to the vector *ClaI* site being opposite that in the original Dmr275c2 plasmid). After transformation of *Escherichia coli*, purified ETS-deletion plasmids were analysed by restriction enzyme digestions and DNA sequencing^{9,14}.

We isolated *Drosophila* clones having 3' deletions to positions +8, +4, +1, -13, -17 and -34; these are shown in Fig. 2.

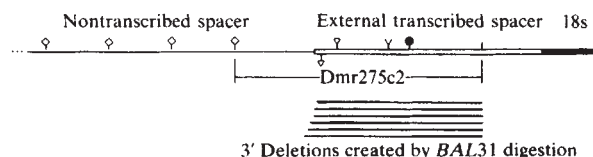


Fig. 1 A map of the region of *D. melanogaster* rDNA that includes the site of transcription initiation. The rDNA segment Dmr275c2 was inserted between the *BamHI* and *HindIII* sites of pBR322 (ref. 9). Bold lines indicate the regions deleted by exonuclease *BAL31* in the cloned rDNA segments that have been studied. The conditions for *BAL31* digestion, and the molecular cloning and sequence analysis of the deleted rDNA fragments, are described in the text and elsewhere⁹. ◇, *AluI*; ▽, *DdeI*; ⊥, *HaeIII*; ●, *HhaI*; Y, *HinfI*; ▽, *TaqI*.

Fig. 2 Primary structure and template activities of cloned *BAL31*-deleted rDNA segments. The sequence of Dmr275c2 from -50 to +30 is shown at the top; beneath it are the *BAL31*-deleted templates described in the text (3'Δ) and elsewhere⁹ (5'Δ). Upper case letters denote rDNA sequences; single underlining shows the NTS and double underlining the ETS. Lower case letters indicate pBR322 sequences. Numbers on the left correspond to the direction and extent of the deletion in a clone, and symbols in the right-hand column indicate the template activity of a clone: ++ means that run-off transcripts are about as abundant as those produced from pDmr275c2; + indicates that transcripts are much less abundant, but are detectable in RNA gels or by S₁ nuclease protection analysis⁹; - means that transcription is not detectable. The 5' deletion segments were characterized in ref. 9.

rDNA clone	-50	+1	+30	Template activity
no Δ	ATGGAATTGAAAATACCGCTTTGAGCAGACGGGTTCAAAACTACTATAGGTAGGCAGTGGTTGCCGACCTCGCATTC			++
3'Δ -34	ATGGAATTGAAAATACCGgaattcttgaagacgaaagggcctcgtgatacgccctattttataggttaatgtcatgataa			-
3'Δ -16	ATGGAATTGAAAATACCGCTTTGAGCAGACGGgaattcttgaagacgaaagggcctcgtgatacgccctattttata			-
3'Δ -13	ATGGAATTGAAAATACCGCTTTGAGCAGACGGCTTcgaattcttgaagacgaaagggcctcgtgatacgccctatttt			-
3'Δ +3	ATGGAATTGAAAATACCGCTTTGAGCAGACGGGTTCAAAACTACTATAggaattcttgaagacgaaagggcctcgtg			+
3'Δ +4	ATGGAATTGAAAATACCGCTTTGAGCAGACGGGTTCAAAACTACTATAGGTggaattcttgaagacgaaagggcctc			++
3'Δ +8	ATGGAATTGAAAATACCGCTTTGAGCAGACGGGTTCAAAACTACTATAGGTAGGCggaattcttgaagacgaaaggg			++
5'Δ -19	tatcacgaggccctttcgtcttcaagaattccGGGTTCAAAACTACTATAGGTAGGCAGTGGTTGCCGACCTCGCATTC			+
5'Δ -27	ggccctttcgtcttcaagaattccAGGACAGCGGGTTCAAAACTACTATAGGTAGGCAGTGGTTGCCGACCTCGCATTC			+
5'Δ -43	gaattccTGAAAATACCGCTTTGAGCAGACGGGTTCAAAACTACTATAGGTAGGCAGTGGTTGCCGACCTCGCATTC			++

Vector/linker sequences adjacent to the deletion segments of rDNA are identical in all cases. Coincidentally, the ligation of an *EcoRI* linker (pGGAATTCC 3') to the terminus of the +1 deletion (5'...CTACTATA OH) restored nucleotides +2 and +3 of the *Drosophila* ETS (transcription initiation is at the underlined A of the sequence 5'...CTACTAT-AGGTAGGC...3' in intact rDNA¹⁵); we therefore treat this clone as a deletion to +3 (Fig. 2). Similarly, the addition of an *EcoRI* linker to the -17 deletion restores nucleotide -16. The deletion plasmids were linearized for run-off transcription by digestion with *PstI*, which cleaves 755 bp downstream of the vector-*Drosophila* rDNA junction (the point of attachment of the *EcoRI* linker). The transcription of 0.5 μg of each linearized deletion clone was evaluated in the *Drosophila* cell-free system. Each transcription reaction also included 0.5 μg of pDmr275c2/*SalI* (the linearized parental, non-deleted clone) as an internal control template to ensure that failure of any deletion plasmid to support *in vitro* transcription was not a peculiarity of a reaction mixture. The *SalI* site is ~980 bp downstream of the transcription initiation site in pDmr275c2 (ref. 9).

Figure 3 shows an autoradiogram of ³²P-RNA isolated from transcription reactions containing linearized DNA templates deleted of sequences by *BAL31* digestion. Templates capable of supporting transcription are expected to produce an ~700-nucleotide RNA (the lower band). Each lane shows the 980-nucleotide run-off transcript from the non-deleted pDmr275c2/*SalI* template, which contains 305 bp of NTS and 680 bp of ETS (the upper band). Deletion of ETS sequences up to +8 or +4 (Fig. 3, lanes 1 and 2, respectively) had little or no effect on the degree of transcription compared with transcription of a non-deleted template (represented by the slower migrating RNA). However, the deletion of sequences to +3 caused a marked reduction in the amount of transcription, as there was little 0.7-kb RNA in lane 3. Templates lacking sequences downstream of -13, -16 or -34 (deleted of all the ETS plus portions of the adjacent NTS) did not support detectable transcription (Fig. 3, lanes 4, 5 and 6, respectively).

Among other organisms, sequences that influence the activity of RNA polymerase I include a region of ~40 bp upstream of the mouse rRNA transcription initiation site¹², and lie between nucleotides -145 and +16 of *Xenopus* rDNA¹⁰. Moreover, sequence homology among three species of *Xenopus* is restricted in this region to the portion -11 to +4, suggesting that this sequence has a major role in the regulation of polymerase I activity¹⁶. Such sequence conservation does not extend to other species that have been examined, including *Drosophila*¹⁵,

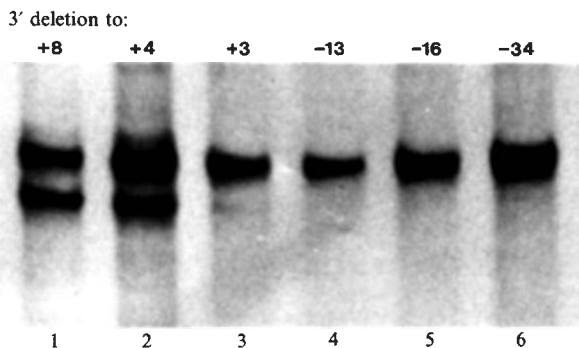


Fig. 3 *In vitro* transcription of 3' deletion templates. RNA labeled by the incorporation of ^{32}P -GMP was purified from transcription reactions, run in a 2% agarose denaturing gel, and visualized by autoradiography⁹. The numbers above the lanes correspond to the extent of the 3' deletion determined by DNA sequencing¹⁴. Each lane shows the 980-nucleotide control transcript (see text). The more rapidly migrating RNA species, when present, was produced from an ETS-deleted template.

yeast¹⁷, and mouse and human¹⁸ rDNAs, but note that in all the species mentioned, rDNA transcription begins with an A residue, and the sequence AGGTA occurs within 10 nucleotides of the initiation site.

A major component of the *Drosophila* polymerase I promoter lies between -43 and -27 (ref. 9), and in this report we have localized additional polymerase I controlling sequences. First, templates that lack all but 3 bp of ETS (Fig. 3), or all but 18 bp of NTS⁹, support low levels of accurate synthesis, thus the region -18 to +3 contains information that directs polymerase to initiate properly, albeit infrequently. We suggest that this region contains a polymerase phasing signal. Second, while the deletion of ETS sequences downstream of +3 markedly reduces the level of *in vitro* transcription, deletion of the ETS up to +4 has little or no effect. This indicates that at least part of a second major component of the polymerase I promoter lies within the first four nucleotides of the rRNA transcription unit. Nucleotide +4, a T residue in the noncoding strand, is evidently an important feature of this 'downstream' portion of the promoter. The 'downstream' promoter sequences are not effective when they are separated from the 'upstream' promoter that involves the sequence -43 to -27, as a template with all but 18 bp of the NTS missing yet containing sequences downstream of -18 (an NTS deletion described in ref. 9 and shown in Fig. 2), supports only low levels of transcription⁹. The 'upstream' portion of the promoter may also not be effective alone, as no transcription is detected from a template that includes the sequence -43 to -27, but lacks the region downstream of -13 (Fig. 3). However, it may well be that the failure of this latter clone as a template is due to the absence of a phasing signal such as the initiation site itself, rather than its lacking the downstream portion of the promoter. In any event, the data presented here indicate the existence of a dual promoter for *Drosophila* RNA polymerase I transcription, in which two components that provide for high levels of *in vitro* transcription are separated by at least 20 bp. One has been mapped using BAL31 deletions of the NTS to the region -43 to -27, and the other has been mapped using deletions of the ETS to the region that includes the base pair +4.

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Clustered illegitimate recombination events in mammalian cells involving very short sequence homologies

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Mammalian cells possess mechanisms that allow unrelated sequences to recombine (illegitimate recombination). This is evidenced by the high rate of recombination between largely non-homologous sequences after DNA transfection^{1,2}. We have analysed the integrated viral sequences present in the polyoma transformed cell line 82-Rat³. Within the single insert of integrated viral sequences there are two regions where multiple recombination events have occurred. The recombination events are particularly interesting as there was no obvious prior selection for their occurrence, and thus they may accurately reflect a normal mechanism of cellular recombination. A total of five recombinant joins have been sequenced. Our results, reported here, indicate that multiple recombinant events occur within small regions (about 50 bp) and that very short homologous stretches (3-4 bp) participate in joining two non-homologous sequences. This suggests that factors other than sequence homologies drive certain recombination events. These results have implications for site-directed recombination following the addition of exogenous DNA.

The structure of the viral insert in 82-Rat cells, as deduced by Southern blotting³, molecular cloning and DNA sequence analysis is shown in Fig. 1b. Viral sequences totalling 1.9 polyoma genome-equivalents are contained in a single insert. Sequences encoding three partial early (early A, B and C) and two partial late regions (late A and B) are present largely in a head-to-tail tandem arrangement. In addition, two segments of DNA are inverted with respect to the body of the viral insert. These inversions identify sites where viral sequences have recombined. The first site (site I) involved an insertion/deletion of viral sequence in early region B. The second site (site II) corresponds to a region where sequences extending from 67 to 94 map units are joined to the body of the viral insert at 49 map units (Fig. 1).

The sequence through the site I insertion/deletion is shown in Fig. 2. Approximately 40 nucleotides of polyoma late region sequences (from nucleotides 3,134-3,131 to 3,079-3,076, late Z) have been inserted into early region B, deleting approximately 230 nucleotides (between nucleotides 1,875-1,878 and 2,114-2,118). The orientation of late Z is inverted with respect to early region B and the body of the viral insert. Recombination between viral sequences appears to have been mediated by homologous stretches of four nucleotides at each end of the insertion/deletion.

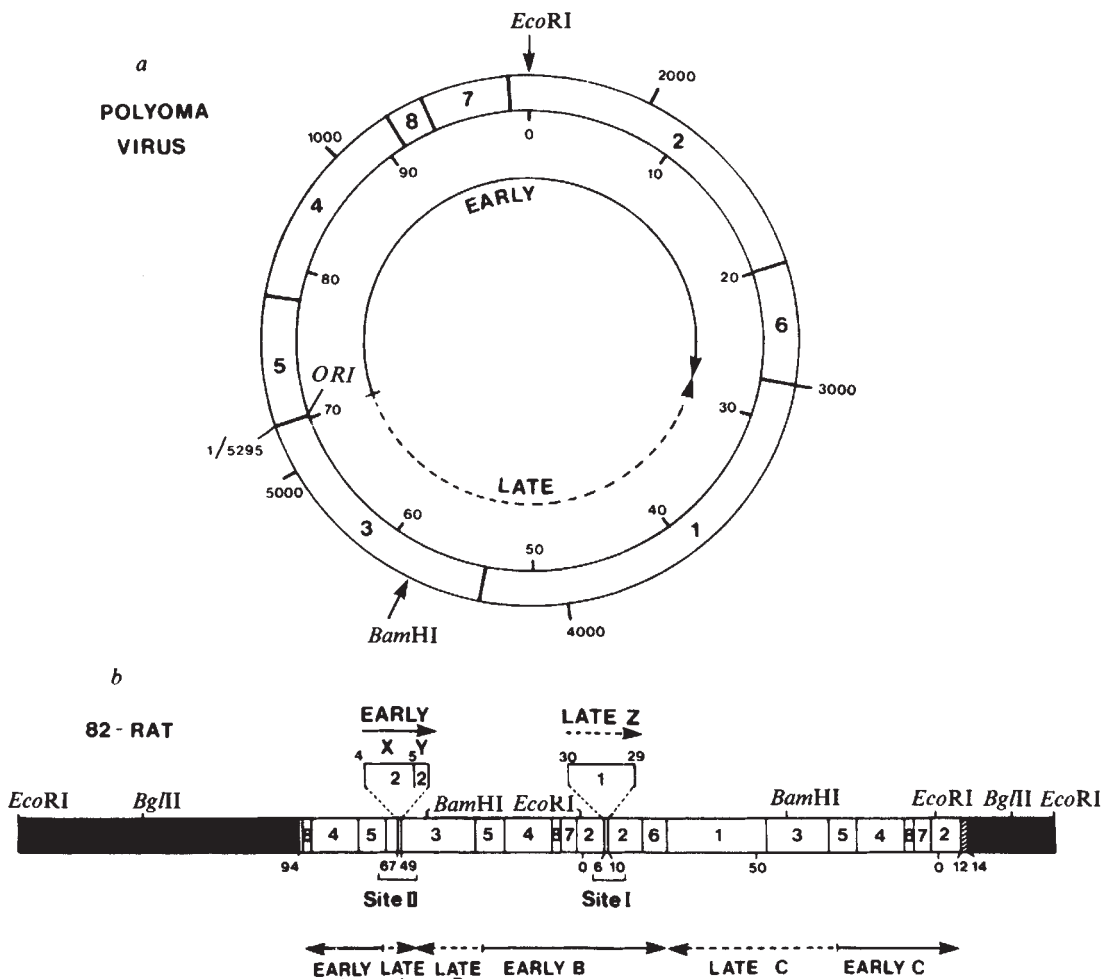
The sequence of site II is given in Fig. 3. Two segments (X and Y), containing polyoma early region sequences, link late regions A and B. Thus three recombination events appear to have taken place within a stretch of 50 nucleotides to join four different segments of polyoma DNA. Overall, the structure of this arrangement is complex. The sequences in late region A are inverted with respect to the body of the viral insert. Early X and Y are not inverted; however, their locations are reversed from their original positions on the polyoma genome (see Fig.

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Fig. 1 Integrated polyoma virus in 82-Rat cells.

a, The circular polyoma virus genome showing the *Hpa*II fragments 1–8. The numbers on the outside of the circle indicate nucleotides starting and terminating at the *Hpa*II-3/*Hpa*II-5 junction where the origin of DNA replication (*ORI*) has been located. The numbers inside the circle indicate map units. The single *Eco*RI and *Bam*HI cleavage sites and the location of early and late transcription are indicated²⁰. **b**, The structure of the integrated viral sequences (white) and flanking cellular DNA (black) in 82-Rat cells as deduced by Southern blotting, molecular cloning and DNA sequence analysis. The hatched area indicates the region in which viral and host sequences are joined (precise junction not yet determined) at the right end of the viral insert. Viral sequences are drawn in relation to the *Hpa*II cleavage map (see **a**) and the direction and extent of viral early and late transcription units are indicated. The numbers on top and below the *Hpa*II map indicate map units (see **a**). Viral sequences comprising 1.9 polyoma genome equivalents are present in a partial head-to-tail tandem arrangement. In addition, the viral insert contains two regions (sites I and II) where viral sequences have recombined. In site I late region Z sequences have recombined with early region B. At this recombination site early region B sequences have been deleted and late region Z sequences are inverted with respect to the body of the viral insert. At site II early region Y sequences have recombined with early region X and late region B sequences, and early region X sequences have recombined with late region A sequences. The presence of inverted sequences containing early late region A was not originally deduced from the Southern blot analysis of cellular DNA, although restriction enzyme sites in the inverted viral sequences were correctly placed³. The presence of the site I insertion/deletion was postulated because of the *S*₁ sensitivity of RNA/DNA hybrids at that site²¹.

Methods: The polyoma transformed cell line, 82-Rat, was isolated as a focus following infection of Rat-1 cells with polyoma as described³. After a sufficient number of generations to isolate cellular DNA (~30), viral sequences were found to be contained in a single insert³. The structure of the integrated viral sequences has remained unaltered for over 2 yr in culture. Subsequently, two *Eco*RI fragments containing portions of the viral insert and flanking cellular DNA were cloned in prokaryotic vectors (Riley *et al.*, manuscript in preparation). One cloned *Eco*RI fragment contained only viral sequences and the other contained viral sequence and the left-hand flanking host sequences.



1). Stretches of homology of three and four nucleotides are found at the recombinant joins between adjacent segments.

It is not clear when the recombination events between viral sequences occurred. However, the present arrangement of viral sequence existed in the original *in vitro* transformed clone following the growth of sufficient cells to isolate DNA (~30 generations³). The larger proportion of viral sequences are present in a head-to-tail tandem arrangement. The mechanism generating tandem structures is not known, but they might arise by homologous recombination or by the integration of replicating molecules^{3,4}. Not all of the sequence arrangements in 82-Rat could have been present on a replicating molecule as the rearrangements detected are not repeated (for example, insertion/deletion in early region B is not present in early region C). Moreover, some of the sequences are derived from viral genomes no longer present in the viral insert (for example, the early region sequences connecting late regions A and B). Thus it is probable that the tandem and inverted sequence arrangements were generated by recombination events either following, or more likely before integration.

Loss of a functional polyoma large T-antigen in *in vitro* transformed cells has been observed to occur either after long-term passage in tissue culture or after formation of tumours by transplantation *in vivo*⁵⁻⁸. The site I recombination events occur in the viral sequences unique to large T-antigen and destroy the coding potential for a full length large T-antigen. But the site I recombination events were detected in 82-Rat cells after short-term passage *in vitro* after the initial transformation event (~30 generations). The site II recombination events occur in the polyoma late region sequences that are not required for transformation and are not known to undergo alterations in transformed cells. The site II events were also present in the 82-Rat cells following growth of sufficient cells to isolate DNA after the initial transformation event³. Thus in 82-Rat cells it would appear that integration has preserved recombination events which did not confer an obvious selective advantage. This is in contrast to other systems where recombination events have been analysed following some selection either in terms of phenotype, ability to replicate or to be packaged into virus particles⁹⁻¹². Although the DNA sequences of the precursors

Fig. 2 Sequence through the site I insertion/deletion. The sequence through the insertion/deletion in early region B is shown above an enlarged portion of the physical map given in Fig. 1b. DNA was 5'-end-labelled at the *TaqI* or *PstI* cleavage sites following treatment with calf intestine alkaline phosphatase and T_4 polynucleotide kinase²². After secondary enzyme cleavage, singly labelled fragments were separated by acrylamide gel electrophoresis, eluted and sequenced by the chemical degradation method of Maxam and Gilbert²³. The sequence of site I in 82-Rat (middle sequence) is compared with sequences derived from the polyoma late region (late A, top sequence) and to polyoma early region sequences (early B, bottom sequence). Homologous nucleotides are indicated by vertical lines and a space (*) is included to improve the alignment of homologous sequences. Early region B extends to nucleotides 1,875–1,878 and is joined to late region Z at nucleotides 3,134–3,131. Late Z extends to nucleotides 3,079–3,076 and joins back to early B at nucleotides 2,114–2,118. Recombination between viral sequences involves homologous stretches of 4 nucleotides at each of the ends of the insertion/deletion. All of the recombinant sequence in 82-Rat cells can be derived from the parental sequences with the exception of the T at nucleotide 3,130, which appears as a C in the previously determined sequence²⁰.

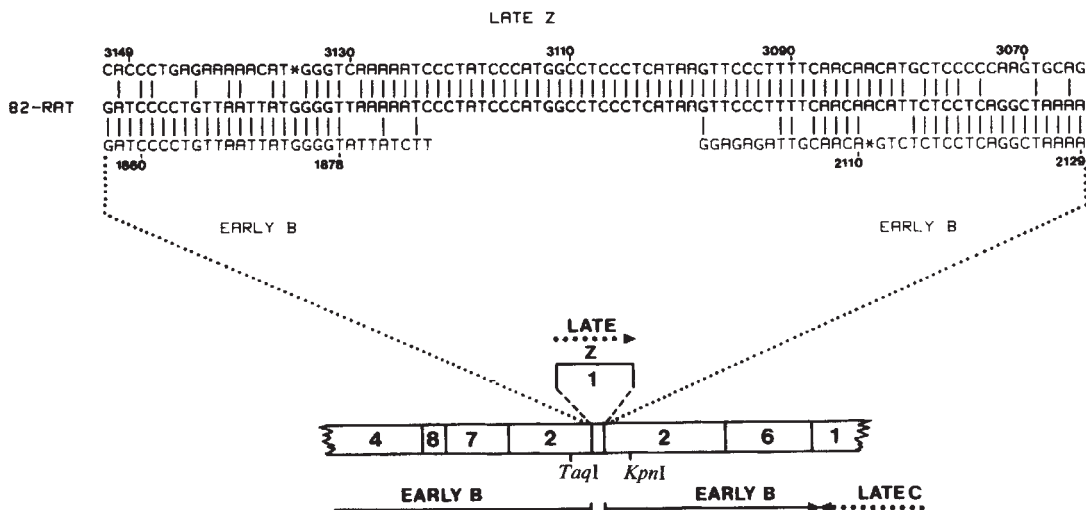
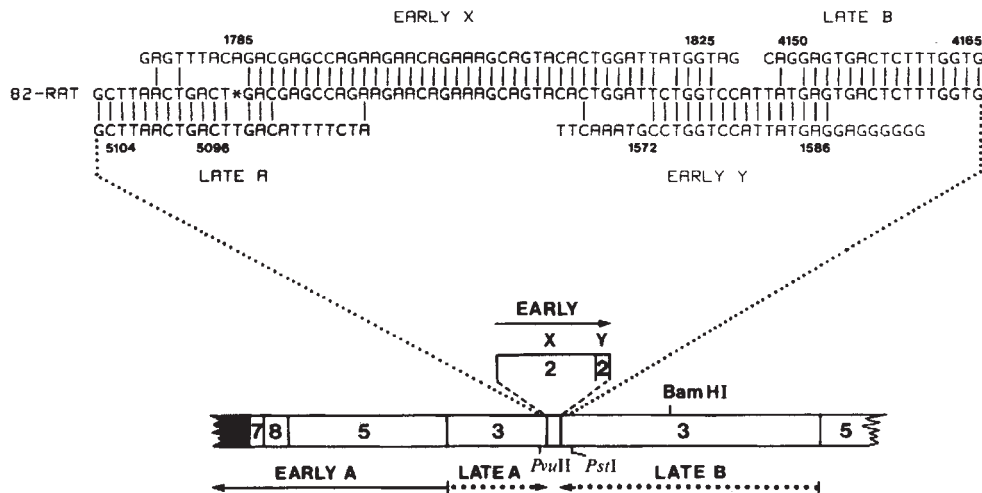


Fig. 3 Nucleotide sequence through the site II recombination. The sequence connecting the inverted early and late regions A and late region B is shown above an enlarged portion of the physical map in Fig. 1b. DNA fragments were 5'-end-labelled at the *PvuII* and *PstI* sites and sequenced as described in Fig. 2 legend. The sequence of site II in 82-Rat (central sequence) is compared with the sequence of different regions of the polyoma A2 virus strain²⁰. Homologous nucleotides are aligned with vertical lines and a space (*) is added to improve this alignment. Analysis of the sequence by the SEQ computer program indicated that two segments of polyoma early region X (top left sequence) and early region Y (bottom right sequence) have recombined to link late regions A (bottom left sequence) and B (top right sequence). The recombination events involved homologous stretches of 3 and 4 nucleotides at the end of each segment. It is possible that the homology to early Y is coincidental, and resulted from a recombination event between early X and late B in which the parental sequences were scrambled. The probability that a match of 15 nucleotides (early region Y) would be found by chance alone in comparing the sequence of 82-Rat with the polyoma genome is approximately 0.001 (ref. 13).



and products of recombination events in 82-Rat cells is precisely known, elucidating mechanisms of recombination remains a difficult task. However, the structure of the joins reveals characteristics which models of illegitimate recombination must accommodate.

First, short homologies are seen at the five junction between otherwise non-homologous sequences. This is the only consistent feature observed. A computer analysis of polyoma DNA against its complement reveals 10 blocks of either 11 base pairs (bp) or 12 bp of perfect homology. We cannot rule out that these regions of homology away from the recombination joins were involved in stabilizing the different strands of polyoma DNA before recombination took place to form the inversions seen. The joins also lack common sequences, structural features or biased base compositions. The participation of short homologies in non-homologous recombination events has also been observed in recombination between viral and cellular DNA both during integration¹⁴ and excision⁹ and in the formation of hybrid and defective viral genomes^{10–12,15,16}.

Second, sequences are lost in the illegitimate recombination events. This is clearly seen in the site I insertion/deletion and would also explain the absence of reciprocal recombination products within the viral insert. Similarly, recombination events in the formation of Adeno-SV40 hybrid viruses, host-substituted variants of SV40 and polyoma and integrated viral DNAs have involved deletions at the sites of recombination^{10–12,14–18}. Again no reciprocal recombination products were found, but in most of these cases, the selection of recombinants precluded detecting reciprocal products even if they were formed.

Third, multiple joins often link distant segments of DNA together within a small region. Such is the case in sites I and II where two and three recombination events have linked sequences together within 50 nucleotides. Similar clustering of recombination events has been previously reported. Thus an inversion of viral sequences in the SV40 transformed cell line 14B is connected to the body of the viral insert by a 41 bp linker¹⁷. Likewise a linker segment of 37 bp connects viral and

cellular sequences at an integration site in a polyoma transformed cell line¹⁸. The origin of these linker segments if not known, hence the number of recombination events cannot be calculated. However, at least two recombination events were required in each case.

All these features point to a mechanism in eukaryotic cells where unrelated sequences are linked in non-reciprocal joining events with loss of sequences. In addition, multiple recombination events are likely to occur within a small region. Despite their widespread occurrence at the site of joining, it seems unlikely that small homologies alone would predispose unrelated duplex molecules to recombine in this manner. Rather it would seem that other events would be required to potentiate recombination. Such events might involve the repair of single-

stranded or broken DNA molecules. This would also explain the high rate of recombination following DNA mediated transfection in which the numbers of broken and possibly single-stranded templates is large. Extrachromosomal DNA may also be much more susceptible to recombination since sequences which have extensively rearranged following entry into the cell are stable after chromosomal integration¹. This type of illegitimate recombination event could interfere with the homologous recombination required for site directed gene therapy of a single copy chromosomal gene following the transfection of DNA into mammalian cells¹⁹.

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A chimaeric antibiotic resistance gene as a selectable marker for plant cell transformation

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The T-DNA region of *Agrobacterium tumefaciens* tumour-inducing plasmids of the nopaline type¹ contains a gene coding for the enzyme nopaline synthase. This gene is expressed constitutively in host plant cells to which it is transferred during tumour induction². We have exploited the regulatory elements of this gene to construct a chimaeric gene that confers antibiotic resistance on transformed plant cells. The chimaeric gene encodes the expected chimaeric transcripts in plant cells, and confers on transformed cells the ability to grow in the presence of normally lethal levels of the antibiotic G418 (ref. 3). Experiments using *in vitro* transformation techniques on single plant cells indicate that this antibiotic resistance can be used as a selectable marker, and can therefore be used in selecting cells transformed by T-DNA vectors that have had the genes for hormone autotrophy deleted⁴. Plant cells transformed by such 'disarmed' T-DNA vectors can be regenerated into entire plants, whose sexual progeny contain unaltered copies of the inciting T-DNA⁵. The availability of this dominant selectable marker should allow a wider range of experiments to be undertaken using different host plants.

The antibiotic resistance gene used in our experiments (*neo*) is derived from Tn5 and codes for neomycin phosphotransferase II. The *neo* coding region, when spliced to viral gene regulatory sequences, confers on animal cells resistance to the aminoglycoside antibiotic, G418 (ref. 3). We have placed the coding region of *neo* under the transcriptional control of the nopaline synthase promoter, which has been identified by DNA sequencing and S₁ nuclease mapping experiments^{6,7}. We included in our construct the 5'-untranslated region of the nopaline synthase gene, but nothing of the 5' end of the coding region. At a position 3' to the *neo* coding region our construct contains the last

portion of the nopaline synthase-coding region, a 3'-untranslated region, and termination and polyadenylation signals.

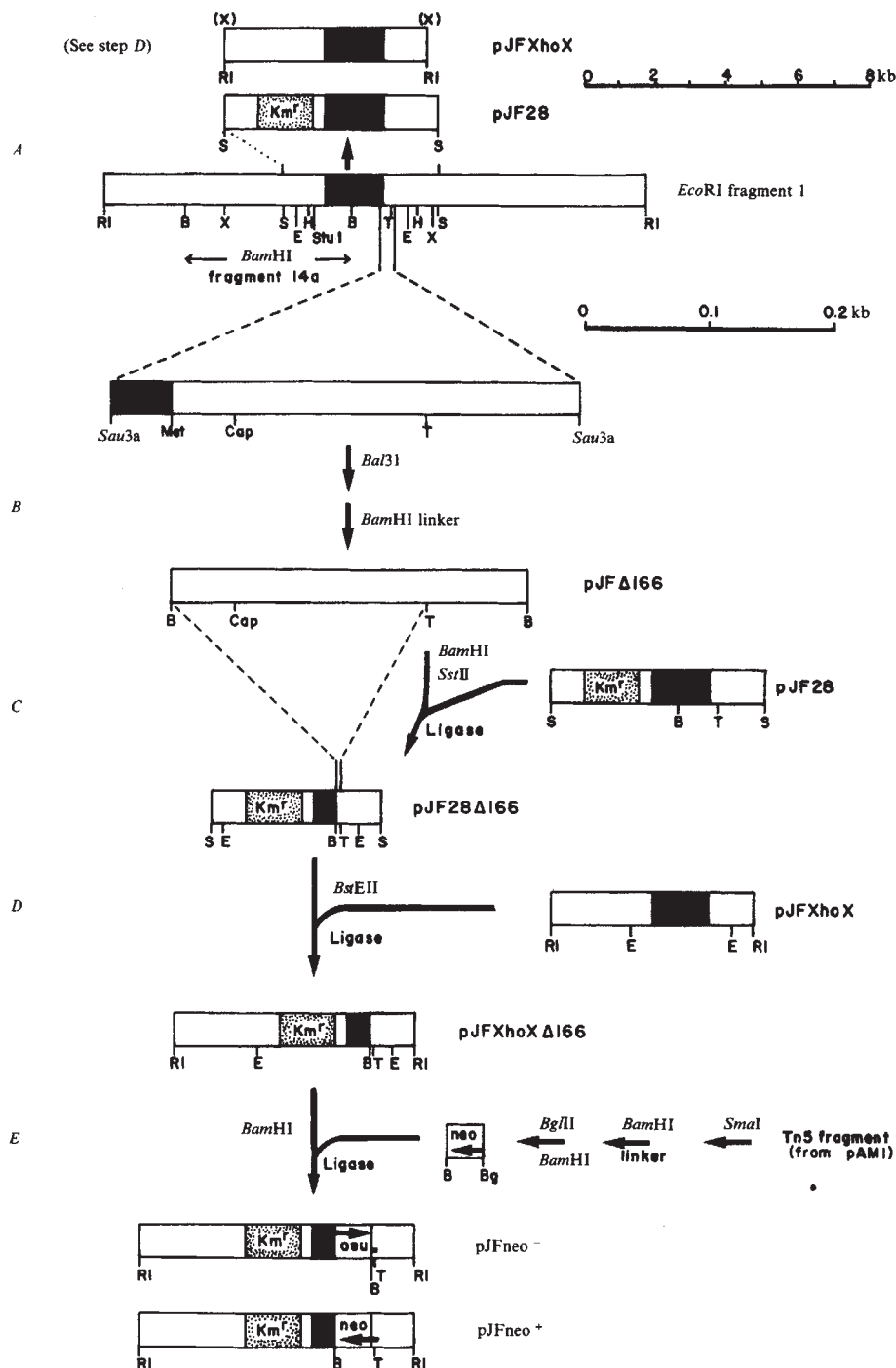
The 5' end of the chimaeric *neo* gene was constructed as outlined in Fig. 1. A 370-base pair (bp) *Sau3a* fragment containing the nopaline synthase promoter region and the first 16 codons of the coding region was treated with *Bal31* to remove the coding region including the initiator methionine codon. The resulting population of molecules was ligated to *Bam*HI linkers and inserted into the *Bam*HI site of pBR322. A 270-bp *Bam*HI fragment with all 48 nucleotides of the nopaline synthase-coding region deleted was identified by DNA sequencing⁸ after subcloning into M13mp7 (ref. 9). The plasmid containing this insert was designated pJFΔ166.

To construct a nopaline synthase gene containing the appropriate deletion and a unique *Bam*HI site for insertion of the *neo* coding region, the 270-bp promoter fragment was digested with *Bam*HI and *Sst*II and cloned into *Bam*HI/*Sst*II-digested pJF28 (see Fig. 1 legend). This produced a deletion of 814 bp in the nopaline synthase gene in the resulting plasmid, pJF28Δ166 (Fig. 1, step C). The resected gene in this plasmid has a unique *Bam*HI site adjacent to the promoter, followed by 423 bp of coding region and the polyadenylation site.

In order to be able to transfer the reconstructed gene to an *Agrobacterium* Ti plasmid by double recombination, it is necessary to have extensive regions of homology on both sides of the gene, and a tightly linked prokaryotic selectable marker¹⁰. We therefore subcloned the deleted nopaline synthase gene, together with the closely linked kanamycin resistance (*Km^r*) marker from pACYC177 (ref. 11), into pJF_{Xho}X to create pJF_{Xho}XΔ166 (Fig. 1, step D).

Construction of the chimaeric *neo* gene started with the plasmid pAM1 (ref. 12), which contains a *Hind*III/*Sal*I fragment of Tn5 (ref. 13). The coding region of the *neo* gene is found on a 1-kilobase (kb) *Bgl*II/*Sma*I fragment¹⁴. To insert this into the deleted nopaline synthase gene, the *Sma*I site adjacent to the 3' end of the *neo* gene was first converted to a *Bam*HI site using linkers to create pAM1 (B). The coding region was isolated from pAM1 (B) by digestion with *Bam*HI and *Bgl*II, and ligated into *Bam*HI-digested pJF_{Xho}XΔ166 (Fig. 1, step E). The orientation of the *neo* coding region in relation to the nopaline synthase promoter was verified in several plasmids by digestion with *Bam*HI and *Sst*II, *Hind*III and *Sst*II, and *Pst*I. Clones with the AUG initiation codon adjacent to the promoter were designated pJF_{neo}⁺, and those in the reverse orientation pJF_{neo}⁻.

Fig. 1 Construction of the chimaeric *neo* gene. **A**, Construction of pJF28, a plasmid containing *Km^r* closely linked to the nopaline synthase gene. A recombinant plasmid containing *Sal*I fragment 10 of pTiT37 was partially digested with *Stu*I, and to this was ligated a 1.5-kb *Hae*II fragment of pACYC177 (ref. 11) (encoding the *Km^r* gene). Plasmid pJF28 contained the *Km^r* insert in the *Stu*I site adjacent to the 3' end of the nopaline synthase gene, as determined by analysis of minipreps. **B**, Isolation of the nopaline synthase promoter. A 370-bp *Sau*3a fragment that contains the first 16 codons of the nopaline synthase gene and extends nearly to the right border of T-DNA was isolated from *Hind*III fragment 23 by preparative gel electrophoresis in 4% polyacrylamide; 5 µg of this fragment was treated with limiting *Bal*31 exonuclease, and the resulting fragments were ligated with 100-fold molar excess of *Bam*HI linkers²⁶ (25 µl, 18 h, 4 °C, 10 U *T₄* DNA ligase). After heating at 70 °C for 10 min the product was digested with *Bam*HI (100 U, 4 h, 37 °C). After preparative electrophoresis (5% polyacrylamide gel), molecules of ~270 bp were excised and recovered by electroelution, DEAE-Sephadex chromatography and ethanol precipitation. This population of molecules was cloned into *Bam*HI-linearized pBR322 (ref. 27). Minipreps of recombinant plasmids (*Ap^rTc^r*) were digested with *Bam*HI and *Sst*II, and analysed for the presence of the desired 192-bp *Bam*HI-*Sst*II fragment. Twelve candidates were subcloned into M13mp7 (ref. 9) and their inserts sequenced using the dideoxy method⁸. One plasmid, pJFΔ166, was found to have been deleted to the nucleotide just 5' to the ATG initiation codon of the nopaline synthase gene. **C**, Deletion of the 5' end of the coding region of the nopaline synthase gene. Plasmid pJF28 (1 µg) and plasmid pJFΔ166 (5 µg) were digested with *Bam*HI and *Sst*II, and ligated together (25 µl, 10 U *T₄* DNA ligase). *Km^r* transformants were screened for the presence of the 192-bp *Bam*HI-*Sst*II fragment. The desired plasmid was designated pJF28Δ166. **D**, Substitution of the truncated nopaline synthase gene into a plasmid containing flanking homology with the Ti plasmid. Plasmid pJFXhoX was made by isolating a 6-kb *Xho*I fragment containing the nopaline synthase gene from *Eco*RI fragment 1, and converting the *Xho*I sites to *Eco*RI sites using molecular linkers²⁶. The desired *Eco*RI fragment of 6 kb was cloned into pBR325-B (ref. 27; pBR325 that has had the *Bam*HI site destroyed by blunting and recircularization). The resulting plasmid, pJFXhoX (1 µg), was digested with *Bst*EII to completion and ligated with 1 µg of *Bst*EII-digested pJF28Δ166 that had been treated with alkaline phosphatase. Substitution in the correct orientation of the kanamycin resistance-conferring *Bst*EII fragment for the *Bst*EII fragment of pJFXhoX was verified by digestion of minipreps with *Bst*EII, *Sma*I and *Eco*RI plus *Bam*HI. The desired recombinant molecule was designated pJFXhoXΔ166. **E**, Construction of chimaeric nopaline synthase/*neo* genes. The coding region of the *neo* gene lies within a *Bgl*II/*Sma*I fragment of Tn5; this fragment includes 30 bp upstream and 180 bp downstream from the coding region¹⁴. Plasmid pAM1 contains a *Hind*III-*Sal*I fragment of Tn5¹² that includes the *neo* gene. The *Sma*I site adjacent to the 3' end of the *neo* gene was converted to a *Bam*HI site using linkers as described²⁶, producing plasmid pAM1(B). The *neo* coding region was excised from pAM1(B) by digestion with *Bam*HI and *Bgl*II and purified. Plasmid pJFXhoXΔ166 (1 µg) was digested to completion with *Bam*HI, and treated with alkaline phosphatase as described above. To this was ligated 0.1 µg of the gel-purified *Bam*HI-*Bgl*II fragment containing the *neo* coding region. Recombinant plasmids with the *Bgl*II-*Bam*HI fusion adjacent to the *Sst*II site were designated pJFneo⁺ and those with the reverse orientation were called pJFneo⁻. The *Escherichia coli* host for DNA constructions was LE392. Drug concentrations used for selective media were: for *E. coli*, ampicillin (*Ap*), 100 µg ml⁻¹; kanamycin (*Km*), 25 µg ml⁻¹; tetracycline (*Tc*), 25 µg ml⁻¹; chloramphenicol (*Cm*), 25 µg ml⁻¹; for *A. tumefaciens* A208, *Km*, 100 µg ml⁻¹; gentamycin, 50 µg ml⁻¹. The scale bar at the top of the figure refers to all constructions except those in step B, which are enlarged (see separate scale bar). Cap is the transcription initiation site, and met the initiation of translation codon. Restriction endonuclease sites used in constructions are designated as follows; B, *Bam*HI; E, *Bst*EII; H, *Hind*III; R1, *Eco*RI; S, *Sal*I; T, *Sst*II; X, *Xho*I; (X), destroyed *Xho*I site. The black box represents the coding region of the nopaline synthase gene. The stippled box represents the prokaryotic *Km^r* gene from pACYC177. The coding region of neomycin phosphotransferase II from Tn5 is designated *neo*, and the arrow on this DNA fragment indicates the orientation of the gene.



To transfer the relevant segments of pJFneo⁺ and pJFneo⁻ to *A. tumefaciens*, the plasmids were digested with *Eco*RI and ligated into *Eco*RI-digested pRK290 (ref. 28). The resulting DNAs were cloned in *E. coli* and subsequently transformed into *A. tumefaciens* strain A208 (ref. 15) using kanamycin selection. Double recombinants (homogenotes) that incorporated the engineered fragment onto pTiT37 were isolated as described previously¹⁰. The structure of the homogenotes was confirmed by Southern blot analysis of total bacterial DNA.

Clones harbouring the desired recombinant pTiT37 plasmids were designated A208neo⁺ and A208neo⁻. These bacteria were used to incite tumours on sterile stem explants of *Nicotiana tabacum* var. Turkish. The resultant tumorous overgrowths were excised, rendered axenic by growth in MS medium¹⁶ containing 500 µg ml⁻¹ carbenicillin, and cloned as described elsewhere¹⁷. Normal teratoma tumour growth occurred 1 month after cloning. To look for the presence of T-DNA, and to determine its structure in the tumours, DNA was isolated

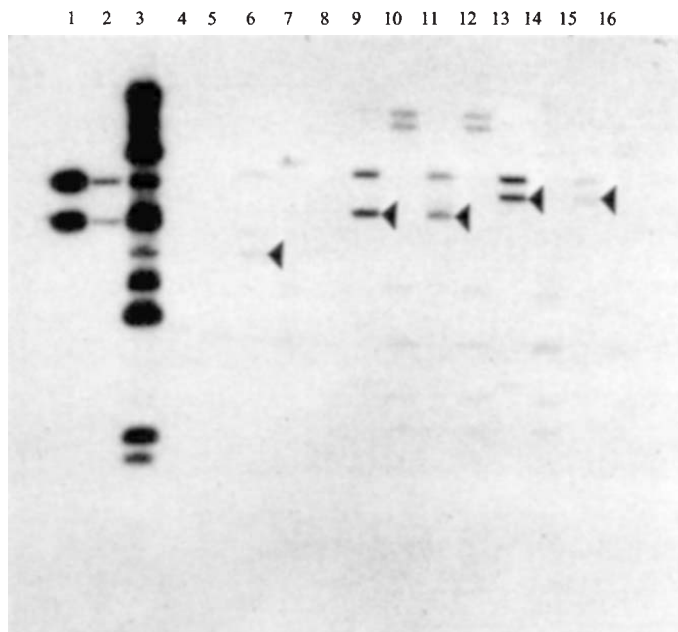


Fig. 2 Southern blot analysis of DNA from cloned tumours. The hybridization probe used is a clone of the entire T-DNA region of pTiT37 cloned in pBR322. Lanes contained the following DNA samples: 1, 10 copy reconstruction; 2, 1 copy reconstruction; 3, molecular weight marker; 4, normal, *Bam*HI; 5, normal, *Eco*RI; 6, HT37, *Bam*HI; 7, HT37, *Eco*RI; 8, no DNA; 9, neo⁺ clone 14, *Bam*HI; 10, neo⁺ clone 14, *Eco*RI; 11, neo⁺ clone 8, *Bam*HI; 12, neo⁺ clone 8, *Eco*RI; 13, neo⁻ clone 2, *Bam*HI; 14, neo⁻ clone 2, *Eco*RI; 15, neo⁻ clone 1, *Bam*HI; 16, neo⁻ clone 1, *Eco*RI. The arrows in the autoradiogram indicate the position of the modified *Bam*HI fragment 14a adjacent to the *neo* gene.

Methods: DNA was isolated¹⁸ from representative cloned tobacco tumour lines incited by A208neo⁺ and A208neo⁻. Positive and negative control DNAs were isolated from HT37-8-70 tumour tissue which contains an unaltered T-DNA insert (G. Jen, unpublished data), and from *N. plumbaginifolia* leaves. Ten µg of each DNA sample were digested to completion with either *Eco*RI or *Bam*HI and electrophoresed through a 0.7% agarose gel. Southern blotting, hybridization and washing were performed as described elsewhere²⁹. Labelled probe DNAs ($1.5-3 \times 10^8$ c.p.m. µg⁻¹) were prepared by nick translation²⁰ using [α^{32} P]dATP (NEN). The molecular weight marker was λ DNA digested with *Eco*RI and *Hind*III, and the reconstruction lanes contained 15 µg and 150 µg of pJFneo⁺ DNA digested with *Eco*RI. The Southern blots were exposed to X-ray film with two intensification screens for 2 days at -70°C.

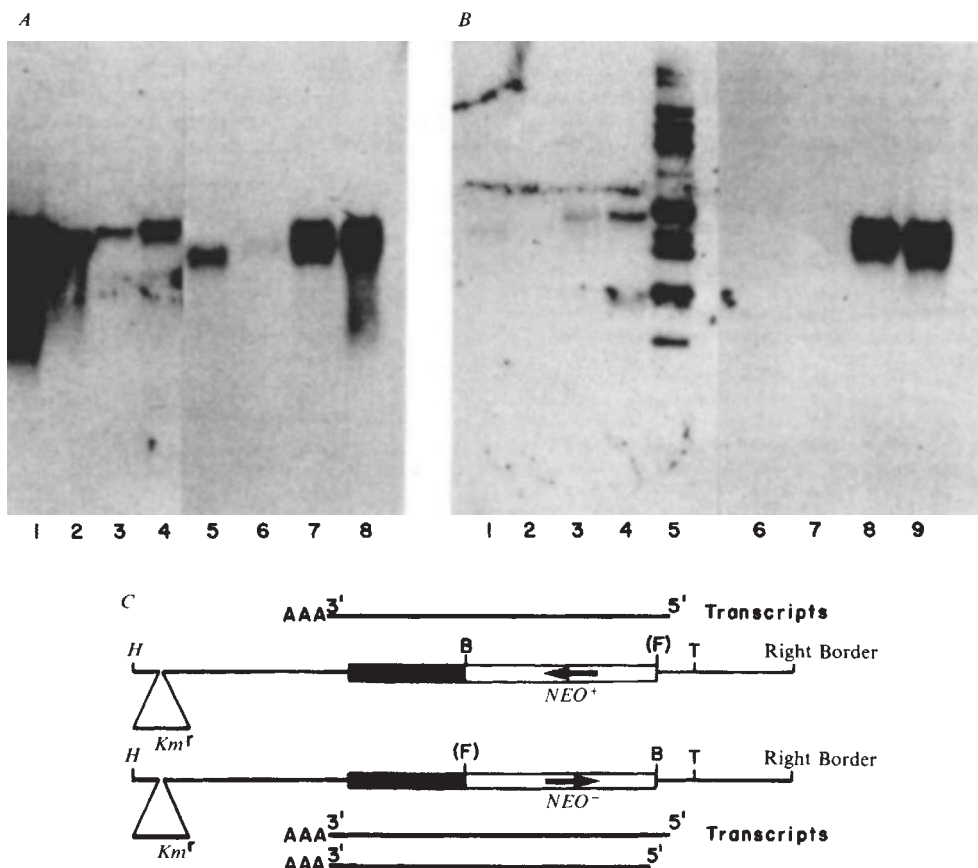
Fig. 3 Northern blot analysis of transcripts. **A**, Hybridization probe specific for 3' end of nopaline synthase gene. Lanes contained the following RNA samples: 1, 20 µg HT37; 2, 10 µg HT37; 3, 5 µg neo⁺ clone 14; 4, 5 µg neo⁺ clone 8; 5, 5 µg HT37; 6, 1 µg HT37; 7, 20 µg neo⁻ clone 2; 8, 20 µg neo⁻ clone 2. **B**, Hybridization probe specific for *neo* coding region. Lanes contained the following RNA samples: 1, 20 µg HT37; 2, 10 µg HT37; 3, 5 µg neo⁺ clone 14; 4, 5 µg neo⁺ clone 8; 5, molecular weight marker; 6, 5 µg HT37; 7, 1 µg HT37; 8, 20 µg neo⁻ clone 2; 9, 20 µg neo⁻ clone 1. **C**, Structure and transcription of chimaeric genes. (F) is the *Bam*HI-*Bgl*II fusion. Lanes 1-4 of **A** and 1-5 of **B** are from the same gel, and lanes 5-8 of **A** and 6-9 of **B** are from another gel. Direct size comparisons between these different gels was not possible. Transcript sizes were calculated from standards run in each gel. The diagram showing a truncated chimaeric transcript in neo⁻ tumours shows only one possible RNA molecule. Another possible transcript may be truncated at the 3' end.

Methods: RNA was isolated from the four tumour clones whose DNA was analysed by Southern blotting (Fig. 2). Control RNA was isolated from HT37-8-70 tumour tissue.

After purification by a guanidine thiocyanate procedure²⁴, poly(A)-containing RNA was selected by oligo-dT cellulose chromatography³⁰. RNA was denatured and electrophoresed in a 1.5% agarose-formaldehyde gel²², and transferred to nitrocellulose²³. Hybridization probes were nick-translated *Bam*HI-*Bgl*II fragment containing the *neo* coding region excised from pAM1(B) and a 1.1-kb *Bam*HI-*Hind*III fragment complementary to the 3' end of the nopaline synthase gene (see Fig. 1 for location). Molecular weight markers were *Eco*RI and *Hind*III fragments of λ DNA that had been end-labelled with [α^{32} P]dATP using Klenow polymerase. The blot was hybridized for 16 h at 41°C, washed as described²⁹, and exposed to X-ray film at -70°C for 2 days with two intensification screens.

from representative tobacco clones¹⁸, digested with *Eco*RI or *Bam*HI and Southern-blotted¹⁹. We used nick-translated²⁰ MINI-Ti plasmid¹² containing the entire T-DNA of pTiT37 (ref. 21) as a hybridization probe for analysis of T-DNA structure. An autoradiogram showing the pattern of hybridization is presented in Fig. 2. Hybridization with MINI-Ti revealed

that our tumour lines contained 1-2 copies of T-DNA identical to that of natural HT37 tumour cells except for the size of *Bam*HI fragment 14a and the diverse border fragments. In HT37 tumours *Bam*HI fragment 14a is 4.5 kb (arrowed in Fig. 2). As expected, in neo⁺ tumours, the corresponding fragment is nearly 6 kb due to the presence of the 1.5-kb pACYC177



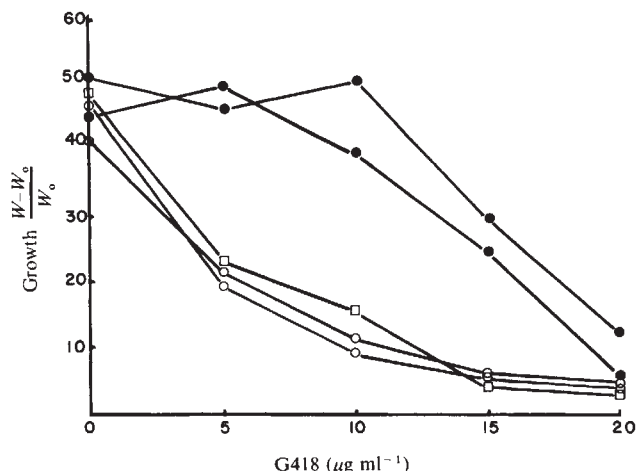


Fig. 4 Growth of tumours on G418-containing medium. Open circles, neo⁻ clone 1 and neo⁻ clone 2 tumours; closed circles, neo⁺ clone 8 and neo⁺ clone 14 tumours; open squares, HT37-8-70 tumour. W_0 , initial weight (30 mg); W , final weight after 25 days.

Methods: Approximately 20 clones of neo⁻ and neo⁺ tumours were analysed for growth on MS medium¹⁶ containing 3% sucrose and various concentrations of G418. Ten pieces of tissue from each clone, each weighing ~30 mg, were transferred to the indicated concentrations of G418 and incubated at 25 °C in a 12 h light/dark cycle for 30 days. Tissues were collected and weighed, and the mean weight increase calculated. The data presented here are for the cloned tumour lines whose T-DNA and transcripts are described above (Figs 2 and 3). The remaining 18 clones of each type (neo⁺ and neo⁻) gave similar data.

synthase transcript. A diagram of the transcription of neo⁺ and neo⁻ genes is presented in Fig. 3C.

Having established that the chimaeric *neo* gene is transcribed in the tumours, we investigated its effect on the plant cell phenotype. The growth rates of neo⁺ and neo⁻ cloned tumours were determined on MS medium without phytohormones, but containing various concentrations of G418. Tumour explants known to contain T-DNA were collected and weighed after 30 days. Figure 4 shows the results obtained for two neo⁺ and two neo⁻ clones. Tumours containing the neo⁻ construction exhibited almost complete cessation of growth on medium containing more than 5 $\mu\text{g ml}^{-1}$ G418, as did natural HT37 tumours. In contrast, tumours harbouring the neo⁺ gene showed no decrease in growth rate until 15–20 $\mu\text{g ml}^{-1}$ G418. We tested approximately 20 additional clones of neo⁻ and neo⁺ tumours, and these displayed almost identical growth curves to their counterparts in Fig. 4. As the growth rate of tumours is strictly dependent on the orientation of the coding region relative to the nopaline synthase promoter, we conclude that the observed differences in growth rate are due to differences in the activity of the *neo* gene in these tumours.

The results presented here demonstrate that a chimaeric gene composed of T-DNA regulatory, bacterial coding and T-DNA termination elements is transcribed in plant cells, and that the expected enzyme activity associated with the translation of the chimaeric transcript is manifest by an enhanced resistance of those plant cells to the antibiotic G418. Preliminary cocultivation experiments²⁵ using *Nicotiana plumbaginifolia* and A208neo⁺ and A208neo⁻ indicate that transformants can be selected using 10 $\mu\text{g ml}^{-1}$ G418 in the growth medium of the regenerating cells (data not shown). Thus, this G418 resistance will be useful in the selection of plant cells transformed by 'disarmed' T-DNA vectors containing no natural selectable marker⁴.

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Note added in proof: Recently Herrera-Estrella *et al.*³¹ have demonstrated the expression of chloramphenicol transacetylase from nopaline synthase gene constructions nearly identical to those reported here.

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kanamycin resistance gene (arrowed in Fig. 2). In neo⁻ tumours, the counterpart of *Bam*HI fragment 14a is 7 kb due to the insertion of the *Bgl*II/*Bam*HI coding region of *neo* (arrowed).

The transcriptional activity of the chimaeric gene in the cloned tobacco tumours was studied by northern blot analysis^{22,23}. Polyadenylated RNA isolated from HT37, neo⁺ and neo⁻ tumours²⁴ was denatured, subjected to electrophoresis and transferred to nitrocellulose. The RNA blots were hybridized with the *neo* coding region fragment, which hybridized to a 1,800-base transcript in neo⁺ tumours (Fig. 3B, lanes 3 and 4) and to 1,700- and 1,800-base transcripts in neo⁻ tumours (Fig. 3B, lanes 8 and 9). As expected, this probe did not hybridize significantly to HT37 RNA (Fig. 3B, lanes 1, 2, 6 and 7). A 1.1-kb *Bam*HI/*Hind*III fragment homologous to the 3' end of the nopaline synthase gene^{6,7} also hybridized to a 1,800-base transcript in neo⁺ tumours (Fig. 3A, lanes 3 and 4) and to 1,700- and 1,800-base transcripts in neo⁻ tumours (Fig. 3A, lanes 7 and 8). This probe, as expected, hybridized to an unaltered 1,600-base nopaline synthase transcript in HT37 RNA (Fig. 3A, lanes 1, 2, 5 and 6). The 1,800-base transcript observed in neo⁺ and neo⁻ tumours that hybridized to both probes is expected of a transcript that originates at the normal nopaline synthase initiation (cap) site, traverses the 1,010-bp *Bgl*II/*Bam*HI *neo* coding region (which replaces 814 bp of the nopaline synthase gene), and terminates at the nopaline synthase polyadenylation site. As the 1,700-base transcript observed in neo⁻ tumours is polyadenylated, it seems likely that it arises from either aberrant initiation of transcription 100 bp from the normal cap site or polyadenylation at the unused poly(A) signal observed 100 bp upstream of the 3' end⁷.

The abundance of neo⁻ and neo⁺ transcripts relative to the natural nopaline synthase transcript can be estimated roughly from these hybridization data. When the different levels of contamination with rRNA are taken into account (visible in ethidium bromide-stained gels), the neo⁺ transcript and both neo⁻ transcripts are approximately as abundant as the nopaline

Is a photon amplifier always polarization dependent?

WITH the help of an ingeniously simple argument, Wootters and Zurek¹ have drawn attention to the fact that there exists no amplifying apparatus such as one or more excited atoms, for example, which will 'clone' an incident photon of arbitrary polarization. More precisely, if $|1_{\epsilon_1}\rangle$ is a one-photon state of polarization characterized by some complex unit vector ϵ_1 , a photon amplifier cannot always turn this into the state $|2_{\epsilon_1}\rangle$ for an arbitrary ϵ_1 . In general, the two-photon state will be some superposition of states $|2_{\epsilon_1}, 0_{\epsilon_2}\rangle$ and $|1_{\epsilon_1}, 1_{\epsilon_2}\rangle$, where ϵ_1, ϵ_2 are orthogonal unit polarization vectors, or even a mixture of states. However, the conclusion of Wootters and Zurek should not be misinterpreted to mean that the output of a photon amplifier has to be polarization dependent.

If the amplifier is in the form of an excited two-level atom in the state $|+\rangle$, with transition dipole moment μ , then the amplitude of the two-photon state $|2_{\epsilon_1}, 0_{\epsilon_2}\rangle$ depends on the scalar product $\mu \cdot \epsilon_1^*$, and would even vanish if the dipole moment were orthogonal to the polarization of the incoming photon. In these conditions there would be no stimulated emission at all, only spontaneous emission. This is apparent if we write for the final state after a short interaction time Δt in the interaction picture

$$|\Psi_{\text{final}}\rangle = \exp(-i\hat{H}_I\Delta t/\hbar)|1_{\epsilon_1}, 0_{\epsilon_2}\rangle|+\rangle \quad (1)$$

with an electric dipole interaction

$$\hat{H}_I = g \sum_{s=1}^2 [\mu \cdot \epsilon_s^* \hat{\sigma}^{(-)} \hat{a}_s^\dagger + hc] \quad (2)$$

We have limited ourselves to a Hilbert space (where the operators are distinguished by the caret $\hat{}$) with just two resonant plane wave modes, and have written $\hat{\sigma}^{(-)}$ and $\hat{a}_s^\dagger$ for the atomic and field lowering operators. From equations (1) and (2) one finds immediately, after tracing over atomic variables, that after a short time Δt the resulting two-photon state is of the form

$$|\Phi\rangle = \frac{\sqrt{2}\mu \cdot \epsilon_1^* |2_{\epsilon_1}, 0_{\epsilon_2}\rangle + \mu \cdot \epsilon_2^* |1_{\epsilon_1}, 1_{\epsilon_2}\rangle}{(2|\mu \cdot \epsilon_1^*|^2 + |\mu \cdot \epsilon_2^*|^2)^{1/2}} \quad (3)$$

The first term is attributable to stimulated emission and the second to spontaneous emission into the other mode. Clearly $|\Phi\rangle$ becomes $|2_{\epsilon_1}, 0_{\epsilon_2}\rangle$ only when the dipole moment μ is parallel to the polarization ϵ_1 , and it becomes $|1_{\epsilon_1}, 1_{\epsilon_2}\rangle$ when μ is orthogonal to ϵ_1 . In other words, for this simple one-atom amplifier the final state depends on the polarization of the incoming state, as Wootters and Zurek have pointed out.

However, lest it be thought that it is the sensitivity to polarization that is the essential element in preventing cloning of the incident photon, we now show that it is not difficult, at least in principle, to construct an amplifier whose output is independent of the polarization. For this purpose we consider a system of two resonant, excited atoms with orthogonal transition dipole moments $\mu_a = |\mu| \epsilon_a$, $\mu_b = |\mu| \epsilon_b$, where ϵ_a, ϵ_b are complex, orthogonal unit polarization vectors. We will not go into the non-trivial question how such a state can be produced in practice, but it might perhaps be done by exposing the atoms separately to different light beams and then bringing them together. The atoms are assumed to be sufficiently close that they experience the same field. Then the interaction may be taken to be of the form

$$\hat{H}_I = g \sum_{s=1}^2 (\hat{\sigma}_a^{(-)} \mu_a + \hat{\sigma}_b^{(-)} \mu_b) \cdot \epsilon_s^* \hat{a}_s^\dagger + hc \quad (4)$$

and equation (1) leads to the following (unnormalized) two-photon state

$$\begin{aligned} &|-\rangle_a, |+\rangle_b [\sqrt{2}\mu_a \cdot \epsilon_1^* |2_{\epsilon_1}, 0_{\epsilon_2}\rangle \\ &\quad + \mu_a \cdot \epsilon_2^* |1_{\epsilon_1}, 1_{\epsilon_2}\rangle] \\ &+ |+\rangle_a, |-\rangle_b [\sqrt{2}\mu_b \cdot \epsilon_1^* |2_{\epsilon_1}, 0_{\epsilon_2}\rangle \\ &\quad + \mu_b \cdot \epsilon_2^* |1_{\epsilon_1}, 1_{\epsilon_2}\rangle] \quad (5) \end{aligned}$$

After tracing over atomic variables we encounter a mixed two-photon state, with density operator

$$\hat{\rho} = \frac{2}{3} |2_{\epsilon_1}, 0_{\epsilon_2}\rangle \langle 2_{\epsilon_1}, 0_{\epsilon_2}| + \frac{1}{3} |1_{\epsilon_1}, 1_{\epsilon_2}\rangle \langle 1_{\epsilon_1}, 1_{\epsilon_2}| \quad (6)$$

This is independent of the polarization of the incident photon and of the two atomic transition dipole moments, so long as they are orthogonal. The first term evidently corresponds to stimulated emission, and the state $|2_{\epsilon_1}, 0_{\epsilon_2}\rangle$ is twice as probable as $|1_{\epsilon_1}, 1_{\epsilon_2}\rangle$, which is attributable to spontaneous emission. There is no cloning, and the general conclusion of Wootters and Zurek¹ is, of course, borne out. But the essential element that prevents cloning is here seen to be the spontaneous emission, rather than any dependence of amplifier gain on polarization. A similar conclusion was also reached in another connection by Milonni and Hardies².

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On replicating photons

WOOTTERS AND ZUREK¹ have recently considered whether it is possible to build a quantum mechanical device which will simply duplicate an arbitrarily polarized incoming photon. They consider two possible situations. In the first, the final state of the device depends on the polarization of the photon. In this case, a photon beam of arbitrary polarization will give rise to a mixed, rather than a pure, final state and will therefore not be properly replicated.

In the second situation the final state of the replicator is considered to be independent of the photon polarization. The authors (as also in a subsequent paper by Dieks²) demonstrate an inconsistency in the quantum mechanical description of this situation which leads them to conclude that in it, too, photon replication is impossible to achieve. However, this second situation is unphysical for a rather serious reason: if the final state of the replicator is independent of the photon polarization, then angular momentum conservation is violated. Photons of different polarization are in different spin states (or different linear combinations of spin states). Thus the polarization of the emitted photon must affect the final angular momentum state of the replicator which emits it.

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WOOTTERS AND ZUREK REPLY—Bussey points out that if the amplifier's final state were independent of polarization, then angular momentum would not be conserved. The question of angular momentum conservation is, however, more subtle than it may seem at first, as the following example shows. (This example is related to work of Wigner, Araki and Yanase on the limitations imposed by conservation laws on the accuracy of measurements¹⁻⁴.)

Let $|l\rangle$ be a certain state of the amplifier which is an eigenstate of L_z with eigenvalue l , L_z being the component of angular momentum along the direction of motion of the photon. Assume that when a right- or left-handed circularly polarized photon interacts with the amplifier in this state, the following angular-momentum-conserving transformation occurs:

$$\begin{aligned} &|\text{one right-handed photon}\rangle \otimes |l\rangle \rightarrow \\ &|\text{two right-handed photons}\rangle \otimes |l-1\rangle \end{aligned}$$

|one left-handed photon> $\otimes$ $|l\rangle \rightarrow$

|two left-handed photons> $\otimes$ $|l+1\rangle$

Now suppose we take as our 'ready' state of the amplifier the state

$$|A_0\rangle \propto \sum_{l=-\infty}^{\infty} e^{-\alpha|l|} |l\rangle$$

Then it is a simple matter to show that for small α , the final state of the amplifier is practically independent of the polarization of the incoming photon, even though angular momentum is conserved. Indeed, the inner product between the two possible pure final states of the amplifier is $\exp(-2\alpha)$, which can be made arbitrarily close to unity. Thus, if angular momentum conservation were the only constraint, then the second situation we consider (that is, cloning with the final state independent of polarization) could in principle still be achieved to an arbitrarily good approximation. This is why it was necessary to give the argument based on the linearity of quantum mechanics, which clearly rules out this sort of cloning. Furthermore, the argument we gave can be generalized to apply to other quantum systems, for which angular momentum conservation may not be a relevant factor.

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Morphology of iodine-doped polyacetylene

THE extensive current interest in polyacetylene, $(CH)_x$, has been stimulated by the discovery that doping with donors or acceptors results in up to 12 orders of magnitude increase in the conductivity and the formation of the metallic state¹. Many aspects of this system remain controversial, including the mechanism for doping and the insulator-metal transition¹, charge transport¹, magnetic phenomena¹ and the fundamental crystal structure¹⁻⁴. The material synthesized utilizing the Shirakawa catalyst is fibrillar as grown, with 200-500 Å diameter fibrils⁵. The fibrillar morphology of this polymer has a central role in doping and compensation. The presence of these fibrils is important for rapid electro-

chemical doping and for potential battery applications⁶. Other morphologies have been suggested to result from polymerization in other conditions¹.

The detailed evolution of the fibrillar morphology with doping is very important for understanding the chemistry of doping and its reversibility as well as mechanisms for charge transport in the polymer. It was recently reported⁷ that the fibrillar morphology changes on doping with iodine^{7,8}. Initial doping was stated to result in the formation of aggregates followed by a uniform melt of various globular features at high doping levels. Contradictory observations that only swelling of the polyacetylene fibrils occurs on iodine doping were reported by another group^{9,10}. (In the studies of refs 9 and 10, 60:40 Au: Pd was used to overcoat the films examined.)

To resolve these differences, we have examined the role of surface versus cross-sectional morphology, the effects of tearing on the apparent cross-sectional morphology, as well as the effects of contrast in scanning electron microscope (SEM) photographs and the ramifications of the choice of coating deposited on the sample to reduce the charging effects. We were able to create the reported SEM patterns at will, and we conclude that the fibrillar morphology only swells on iodine doping, with no gross change in morphology.

Both the surface and cross-section of the film were observed to have the same fibrillar morphology, although sometimes dense matting would obscure the fibrils on the surface. Similarly, it was found that tearing would sometimes lead to drawing together of the fibrils into the featureless bundles reported previously⁷. Use of very high contrast highlights these features, making it even more difficult to observe the actual fibrillar morphology.

The choice of overcoating was observed to lead to very large effects. Without overcoating, the fibrillar morphology of the surface and bulk were clearly observed for $(CHI_{0.08})_x$ and $(CHI_{>0.15})_x$, although sample charging did lead to some distortion of the image. D.c. plasma deposition (100 µm air present) of 80-100 Å of a 60:40 gold:palladium alloy (a composition often used in SEM studies^{11,12}) resulted in a sharper image with no charging. Use of 80-100 Å of evaporated or sputtered gold alone as the overcoating led, however, to an apparent aggregation of the gold on the surface. The resulting films no longer showed their fibrillar morphology. Instead clumps and aggregates were observed. Indeed, under high contrast bright white spots appear and all signs of the underlying fibrils (both surface and cross-section) are suppressed.

Our experiments have therefore enabled us to categorize the effects of surface versus bulk study, sample tearing, SEM contrast and metal deposition¹³. We have determined that the fibrillar structure is maintained with iodine doping although there is fibrillar swelling. There

is no evidence from these studies to support a percolation model of the insulator-metal transition. Sample tearing, high SEM contrast and gold deposition can lead to the observation of extrinsic nonfibrillar morphologies unrelated to the true polyacetylene morphology. Hence, care must be taken in the interpretation of any SEM data. All relevant conditions for the SEM studies must be known in order to interpret the micrographs properly.

We acknowledge discussion with, and the cooperation of, A. G. MacDiarmid and A. J. Heeger. Work at the University of Pennsylvania (T.W. and M.A.) was supported by NSF grant no. DMR 80-22870. Part of this study was performed while M.A. was at Laboratoire de Chimie Macromoléculaire, Université des Sciences et Techniques du Languedoc.

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ROLLAND AND CADENE REPLY—Following Epstein *et al.*'s discussion of the morphology of iodine-doped $(CH)_x$, we would like to make some comments based on results of new experiments that we have carried out.

Epstein *et al.* assume that the 'white points' reported in our paper¹ are artefacts produced by gold sputtering alone, because gold aggregates also appear on undoped (CH)_x.

However, we have never observed white points on undoped gold coated material in any of our experiments. We have examined the effects of three iodine doping methods:

(1) Liquid-phase (I₂ in pentane solution) doping; (2) vapour-phase doping at room temperature; (3) vapour-phase doping at low temperature (Epstein method²), associated with several techniques for excess dopant desorption:

- (i) liquid phase desorption (pentane);
- (ii) dynamical pumping (10⁻² torr);
- (iii) Epstein method.

Using the same 80-Å gold coating alone, the aspect of the doped samples, obtained by these various methods, is different.

With the method (2ii), we nearly always observe 'white points', with size increasing according to the dopant level. When the sample is dedoped using method (i) we notice a more homogeneous fibrillar aspect. Using the Epstein process (3iii), we generally do not observe large white points.

Consequently we think that the different observations may be due to the various different ways of preparing the experimental samples. Taking into account that the gold-coated undoped (CH), do not show white points, the gold coating is not responsible for the modifications related to the iodine presence. As the largest white points distribution appears when using the roughest doping and dedoping method (2ii), we can assume that the iodine distribution is often inhomogeneous (corroborated by our transmission electronic microscopy observations³). On the other hand, rough pumping can produce a local desorption of iodine with a fibril bursting and iodine heaps appearing on the surface.

Gold may be preferably deposited on the highly-iodinated sites and consequently could exhibit the inhomogeneous iodine distribution.

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Note added in proof by Rommelmann et al.: Secondary electron yield is a weak erratic function of atomic (*Z*) number¹⁴ compared with its strong dependence on surface topography. It is, therefore, incorrect to conclude that white areas on a highly structured surface, such as cross-sections of polyacetylene samples, are due to the high *Z* number of the dopant moiety. As has been shown in detail in ref. 13 these white areas are either agglomerates of coating particles and/or fibrils (or bundles of fibrils) being viewed end-on under extremely high contrast. As we have concluded previously^{9,10,13}, the diameter of these fibrils may be a function of dopant and doping procedure.

Furthermore, the transmission electron microscope results referred to by Rolland and Cadene are not under question, nor are they an appropriate corroboration as the inhomogeneities reported occur on a much smaller scale than may be resolved using SEM.

Asymmetric ligand binding by haemoglobin

WEBER has presented an interesting hypothesis about the origins of the asymmetry of the oxygenation curves of human haemoglobin¹. However, it is important to point out the significance of an assumption that is essential to his analysis—that the energetics of a boundary or interface are defined only by the presence or absence of ligand on the subunits involved in the interface. Constraints which are termed third and fourth order are specifically ignored. Weber fails to make note of the fact that this assumption excludes consideration of all models that invoke concerted transitions in quaternary structure. Such models permit asymmetric ligand binding without the need for dissimilar subunits. The two-state model of Monod *et al.*² is intrinsically asymmetric, being symmetric only in the special case when $1/L = c^{n/2}$, where *n* is equal to the number of binding sites per molecule. For a tetrameric structure it is positively asymmetric when $1/L < c^2$. If more than one quaternary state is possible, the free energy of interaction between two subunits will depend not only on the presence or absence of ligand on these subunits, but also on the particular quaternary state of the entire molecule. This is the equivalent of saying that the constants *K*_{BB} and *K*_{AB} of the square model of Koshland *et al.*³ will have as many different values as there are quaternary states. Such a feature, when added to the sequential model of these authors, introduces asymmetry even when the subunits are identical.

Thus the conclusions reached by Weber, although valid for systems that are properly described by simple sequential mechanisms, are not necessarily true for all systems. As they lack such general-

ity, it remains uncertain that the presence of dissimilar subunits in the haemoglobin molecule is a necessary condition for asymmetric oxygen binding. Although few would argue that haemoglobin can be properly described by a simple two-state model, the occurrence of alterations in quaternary structure, in addition to sequential changes in the tertiary structures of the subunits, seems very likely.

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WEBER REPLIES—Any phenomenological description of oxygen binding by haemoglobin can accommodate asymmetric titration curves if the variation allowed to the binding constants is sufficiently large. In the two-states description negative, zero or positive binding asymmetry may be accommodated according to whether *Lc*² is, respectively, smaller than, equal to or greater than unity. But, how are *L* and *c* chosen? Certainly not by appeal to any principles that involve the physical characteristics of the system, but because they are the best values in the fitting of experimental data. If Curie's principle¹—as I interpret it—is correct it follows that *Lc*² ≠ 1 shows the existence of asymmetric causes. Failure of Curie's principle would require the observation (not simply the postulation) of systems of identical subunits showing asymmetric binding. Until this is found, I prefer to believe that an asymmetric cause (difference in the diagonal interactions of the subunits) underlies the asymmetric binding by haemoglobin.

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That striking statesman

Robert Fox

Benjamin Franklin: A Biography. By Ronald W. Clark.
Weidenfeld & Nicolson: 1983. Pp.527. £18.50.

THE public face of Benjamin Franklin is a familiar one. It is a face of good-hearted simplicity and sober common sense, qualities which allowed Franklin's natural humility to survive unimpaired as he rose from the obscurity of a Boston printing house to the rank of "the first American", an Enlightenment *philosophe* who could stand proudly before Louis XVI at Versailles wearing neither wig nor sword.

IMAGE
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Ben Franklin, "the first American".

The *Autobiography* in which Franklin handed down a carefully doctored account of his life was understandably a favourite work in the young American republic. Making much of Franklin's modest origins, it impressed the youth of America with the limitless power of self-help and the virtues of abstinence and industry that are epitomized in the most famous of Franklin's literary creations, Poor Richard.

The real Franklin, as might be expected, was very different. There was in fact rather little of the homespun Poor Richard about him. Ronald Clark's richly documented biography shows him whoring with relish as a young man in Philadelphia, indulging in business practices that verged on the unscrupulous, setting up in style in London and at Passy, and revelling in the refined world of the Parisian salons which he frequented for eight years towards the end of the Ancien Régime. During his stay in England as the representative of the aggrieved Pennsylvania Assembly (1757-1762), he was so taken with London society that he was tempted to settle there.

In these years, it seems, he still proudly regarded himself as "a Briton". Indeed, by an irony that Clark exploits to good effect, only his wife's reluctance to follow him across the Atlantic prevented a course of action which might have diverted America from the road that led to Yorktown and independence.

Although this is only incidentally a scientific biography, Clark shows that the discrepancy between myth and reality has extended to Franklin's science. In his own lifetime and for decades afterwards, Franklin was revered as the inventor of bifocal spectacles, the lightning conductor and the Franklin stove, and it is certainly the case that his interest in gadgetry was substantial and abiding. But gadgets, however ingenious, have little to do with Franklin the scientist. Far more relevant to this Franklin is the comparatively slender volume of letters to his London friend Peter Collinson which he published in 1751 as *Experiments and Observations on Electricity*. A clear contemporary assessment of *Experiments and Observations* is implied by the fact that by 1774 it had gone through five English editions and been translated into German and, at Buffon's suggestion, into French. The book also stimulated immediate repetition of the "Philadelphia experiments" throughout Europe, most notably at Marly-la-Ville, where lightning was spectacularly drawn down from the clouds and shown to be a form of electricity, as Franklin maintained.

Clark treats Franklin's science lucidly, though without attempting a serious reinterpretation of either its content or its intellectual and social context. So, at a time when the Yale University Press's *Papers of Benjamin Franklin* are eating an ever greater hole in our library budgets and turning Franklin into one of the best-documented figures in history, the problems of interpretation remain as numerous as ever. It would be intriguing to know, for example, what light the case of Franklin might throw on the supposed provinciality of culture in colonial America. Does correspondence of the kind that Franklin sustained with natural philosophers in Europe imply that he felt America to be dangerously detached from the hub of things, or did the Europeans genuinely feel that Franklin's Philadelphia had something to offer them? It seems clear that by the time of Franklin's death in 1790, European interest in American science was diminishing rapidly, and it remained at a low ebb until its dramatic

revival in our own century. But the circumstances of that decline of interest have still to be elucidated.

Biography is such a notoriously difficult genre that it may be unreasonable to expect even a master of the art like Ronald Clark to confront these wider issues. But issues are what his richly informative study appears, above all, to lack. The book gives little impression of the current interest in the Newtonian tradition in the eighteenth century and in the aether theories which engaged so much of Franklin's attention. In the realm of general history too, there are problems. The bibliography has no place for Henry F. May's outstandingly suggestive book *The Enlightenment in America* or for Donald H. Meyer's *Democratic Enlightenment*. Of course, Clark may disagree with May's ingenious slicing up of the American Enlightenment into phases characterized as moderate, sceptical, revolutionary and didactic. But to ignore secondary literature of such obvious relevance and interest is to court the charge of scholarly myopia. It is a pity to see signs of that malady in a book which, as a purely personal and political biography, nevertheless has a great deal to commend it. □

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Not the end of the world

Joseph Silk

The Ultimate Fate of the Universe.

By Jamal N. Islam.

Cambridge University Press: 1983.

Pp.156. £7.95, \$13.95.

Evolution of the Universe.

By I.D. Novikov.

Cambridge University Press: 1983.

Pp.176. £7.95, \$14.95.

THE Universe is open: it will expand forever. The first threat to civilization, notwithstanding the odds on self-destruction, will come when the Sun evolves into a bloated, red giant star. But this is some five billion years hence, and we may confidently expect that man will long since have civilized other planets around other stars. Even the supply of stars, however, is finite. The resources of energy are not inexhaustible. Stars die, collapsing into black holes, white dwarfs or neutron stars, more compact than the Earth. The supply of gas for making new stars gradually runs down.

Long-range forecasts of our fate in such a far-distant future provide the basis for J.N. Islam's *The Ultimate Fate of the Universe*. Written without the use of mathematics, it is intended for the general reader with little background in astronomy

and particle physics. Islam has succeeded in making the fate of the Universe accessible to a wide audience. He describes many aspects of cosmology, from the present state and structure of the Universe to the evolution of normal stars and the formation and likely death of black holes themselves.

A central theme is that energy remains the key to maintaining life. A truly resourceful civilization will have ample energy supplies available for many aeons. It can mine dead stars, and extract nuclear energy from the stuff out of which many white dwarfs are made, helium, carbon and oxygen. Only when all lighter matter is converted into iron — the ultimate slag heap of the Universe — is the thermonuclear energy supply exhausted. Even then, black holes themselves can be utilized as energy sources if they are spinning. Shoot a particle nearby at the right angle, and it emerges with more energy. All of these energy reserves are limited, however. Nevertheless, one could cope for a very long time, were a more severe catastrophe not to arise. Protons, the essential stuff out of which we are made, may decay. Their decay within 10^{31} or 10^{32} years is predicted by the grand unification schemes, which couple protons and lighter particles.

However, if experiments now underway fail to confirm proton decay, the possibility emerges that the proton is stable. Then our hypothetical civilization of the distant future gains a prolonged lease on life. Many of the stellar remnants in our galaxy and in nearby galaxies will coalesce into a gigantic black hole, under the action of gravitational forces. But even black holes are destined eventually to decay into radiation, according to predictions of the quantum theory of gravity. It will now take some 10^{100} years before all black holes have evaporated. All may not then be lost, since many white dwarfs will remain, abruptly ejected from their parent galaxy by close encounters with other stars to become wanderers in intergalactic space. These are potential reservoirs of energy for many more aeons.

The final fate of all discrete lumps of matter occurs with quantum tunnelling. Spontaneously, any surviving object collapses into a black hole that promptly, relatively speaking, evaporates. It may take a long time, some $10^{10^{26}}$ years in fact, to destroy a white dwarf, but finally every star has vanished. Perhaps consciousness could be maintained in some suitably ethereal form, and all memory of the former glories may not be lost, but the outlook is increasingly bleak.

As this grand scenario for cosmic evolution is developed, Islam carefully explains the interplay between particle physics and cosmology, and there is even an up-to-the-minute chapter on proton stability. The main difficulty likely to be encountered by the reader new to astrophysics is that the book covers too much material in too few pages. But overcoming

this hurdle is well worth the effort. The reward is a rare revelation: the unveiling of the unimaginably remote future of the Universe.

A more challenging discussion of cosmology is given by I.D. Novikov in *Evolution of the Universe*. The scope is less, covering merely the origin of the large-scale structure of the Universe, and the level is higher. Novikov has no hesitation about using a simple equation to illustrate a point that words would only obscure. Nevertheless, many topics are covered in sufficient generality that the average reader should acquire the comforting feeling that he understands the scientific issues which surround topics ranging from Mach's principle to quantum cosmology.

Much of Novikov's discussion has a Soviet perspective. This is not so obtrusive as to be unwelcome, for by and large it seems justified. We learn how Friedmann outmanoeuvred Einstein in developing an expanding model for the Universe. We learn how Novikov himself in 1964 was the first person to take Gamow's hot big bang theory sufficiently seriously and realize that the cosmic background radiation might be experimentally measurable. It was detected the following year by Penzias and Wilson, who were unaware of Novikov's work.

Soviet scientists are playing a major role in developing the modern theory of galaxy formation. Ya. B. Zeldovich, with three Lenin medals in diverse areas of applied physics already to his credit, developed an interest in cosmology and pioneered, in the 1970s, the concept of giant pancakes. These gas clouds contain the mass within a thousand galaxies. They collapse into thin, dense sheets of gas that fragment into galaxies, clusters of galaxies developing where adjacent sheets intersect. Gaping voids are left behind throughout most of space. Only in the past two years has the advent of deep redshift surveys of galaxies furnished impressive, if not conclusive, evidence of vast voids in the galaxy distribution, as well as the presence of thin sheets and elongated filaments of galaxies. All of these features are predicted by the pancake theory of Zeldovich and his collaborators.

Novikov's exposition is selective. It covers the cosmic singularity at the beginning of time and the esoteric concept of particle creation, but omits grand unification and the origin of the specific entropy of the Universe. Novikov is refreshingly frank about our ignorance concerning, for example, what may have preceded the singularity. The going may be a little rough for the uninitiated reader, but Novikov provides an admirable overview of current thinking about the origin of the galaxies. □

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Telling tales

Erik Trinkaus

Wildmen: Yeti, Sasquatch and the Neanderthal Enigma.

By Myra Shackley.

Thames & Hudson: 1983. Pp.192. £7.50.

Published in the United States under the title *Still Living!*, price \$16.95.

RECENT years have seen increasing speculation about human-like creatures that are said to inhabit the remote regions of the Northern Hemisphere. These creatures are known by a variety of names such as Bigfoot, Yeti, Sasquatch and Almas, but universally are described as of human size or larger, hairy, and highly elusive. As the number of reports of these creatures increases, so does the controversy as to whether they exist or are merely folktales passed on by the faithful and gullible. Attempts by anthropologists to clarify the situation, given their training in primate evolutionary biology, therefore should be welcome. The volume under review is such an attempt to appraise the evidence for "wildmen", as Myra Shackley collectively refers to these creatures, and to present an hypothesis as to their identity.

The author claims that she is going to "take a close look at the mass of evidence for living wildmen in order to see whether a convincing pattern emerges" (p.15). One perceives early on, however, that the author's null hypothesis is that these "wildmen" exist. No evidence is presented, if it ever could be, that they do not exist, and therefore most of the accounts of them are accepted as valid. The only pattern that emerges is in the structure of the tales, which probably has more to do with conventions in storytelling than with the creatures under investigation.

Most of the book consists of supposedly critical evaluations of reports of "wildmen" from Europe, north-west North America, the Himalayas, China, Mongolia, the Pamirs and the Caucasus (a chapter each). The existence of European "wildmen" is rejected, since the evidence is considered less than satisfactory. By contrast the reports of them in North America and Asia are taken to be largely accurate, even though a few clearly fallacious or grossly exaggerated accounts are rejected. This portion of the book is well written, but it becomes somewhat monotonous. One is presented with case after case of an individual of high repute (usually of European descent) relaying accounts of encounters with "wildmen" from local people and/or personal experience. The accounts are always at least second-hand (the author never claims to have seen, touched, or heard one of these creatures) and usually third or fourth hand, most of the encounters with "wildmen" having taken place many years before they were written down. There are constant appeals

for the reader to accept the veracity of the observers, even though why one should believe them is unclear.

In Chapter 9, Shackley hypothesizes that the Mongolian "wildmen", the Almas, are a surviving Neanderthal population. This is one of the more interesting parts of the book, since the Neanderthals are a well-known biological group. It therefore provides an opportunity to evaluate the thoroughness of Shackley's scholarship and hence a framework against which to judge her evaluations of "wildmen" stories. What, then, is her "evidence" that the Almas of Mongolia are Neanderthals? It consists entirely of Middle Palaeolithic-like tools in areas of Mongolia (none illustrated or described in detail), which local people say are made by "wildmen" in the nearby mountains. Not only is this shaky evidence upon which to base such an hypothesis, but it bespeaks ignorance of the associations of Upper Pleistocene lithic industries with human biological forms. Shackley is apparently unaware that anatomically modern human beings, as well as Neanderthals, are associated with Middle Palaeolithic industries in the Crimea and the Near East. The presence of such lithics in Mongolia, therefore, does not document the existence of Neanderthals, whatever the geological ages of the implements might be.

In addition, the density of misinformation on the Neanderthals is appalling. It appears that Shackley does not realize that two adult Neanderthal partial skeletons were found at Spy, not one (p.141), that they were excavated in 1886, not about 1866 (p.141), that Boule estimated the cranial capacity of La Chapelle-aux-Saints 1 to be 1,620 cc, not 1,450 cc (p.143), that there is no evidence for rickets or other specific dietary deficiencies in any of the known Neanderthal specimens (p.146), that Neanderthal halluces were of the same relative length as ours (p.148), and that Skhul 5 is not a Neanderthal but a robust, early anatomically modern human (p.159). These may appear to be detailed points of little importance, but they illustrate Shackley's disregard for factual accuracy. All are easily verifiable, unlike her claims about "wildmen".

Shackley's lack of competence in human palaeontology and palaeolithic archaeology does not lead one to have much confidence in her other opinions. Thus, this book is merely another contribution by an individual already convinced that "wildmen" exist. □

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Flora of Australia. The second volume in this series to be released (Vol. 8, *Lecythidales to Batales*) has recently been issued. Price is hbk \$A34, pbk \$A29; postage is \$A5. For review of Vol. 1 in the series and details of the publisher see *Nature* 296, 275; 1982.

Protein in the picture

Wayne A. Hendrickson

Hemoglobin: Structure, Function, Evolution, and Pathology.

By Richard E. Dickerson and Irving Geis. *Benjamin/Cummings: 1983. Pp.176. \$34.95, £22.45.*

MAX PERUTZ describes haemoglobin as the hydrogen atom of biology. Indeed, this protein does serve as the model for a broad reach of biological sciences and has also been the proving ground for countless techniques. Because haemoglobin plays such a central role in science and in life, Dickerson and Geis quite naturally chose it to illustrate principles of protein structure, function and evolution when they set out to revise their first book, *The Structure and Action of Proteins* (Benjamin, 1969). The example became the subject, and the planned revision evolved into *Hemoglobin*.

Readers of the previous book will recognize much of the material in the early stages of this one. However, nearly everything in the last three quarters is fresh, including a beautifully illustrated description of the mechanism of allosteric action in haemoglobin as well as outstanding sections on globin evolution and on abnormal human haemoglobins. In general, the clear exposition and wonderfully comprehensible views here of the three-dimensional structures of haemoglobins and myoglobins provide an excellent complement to the stereographic details in the Fermi and Perutz's *Atlas* (for review see *Nature* 302, 362; 1983).

Molecular evolution is one of Dickerson's major interests and the corresponding section is probably the strongest in the book. Thorough coverage of sequence variability in the globins is backed up by a lucid discussion of mutation rates and evolutionary theory. (It is a pity that Perutz's recent work on species adaptation came too late to be included.) One also finds a fascinating description of haemoglobin gene structure and its relationship to changes in expression during pre- and post-natal development. There is, however, a failing with respect to invertebrate haemoglobins. One gains the impression that the cited monomeric examples are typical when, in fact, giant extracellular haemoglobins are commonplace.

The mutant haemoglobins, a subject closely related to evolution, are also admirably covered. This part of the book flows from the aetiology of thalassaemias to a comprehensive table of known point mutations in human haemoglobin. But the highlight is sickle-cell anaemia. Here Geis's talents as an illustrator again come to the fore. Both the text and the figures convey a

marvellous picture of the molecular basis of this disease and the hope for a cure.

The metaphor that likens haemoglobin to the hydrogen atom is apt, in large measure, because so much is known about the molecular structure of this protein. But the wealth of information in a three-dimensional structure must be comprehended for it to have an impact. This book makes that possible for haemoglobin. Dickerson's enviable ease with words and Geis's word-saving pictures clear away the clutter and focus our attention on essentials. Consequently, we see fields ranging from evolution to medicine illuminated by the structure of haemoglobin. We await more in the same vein from this outstanding partnership when they reach the original goal of updating their 1969 book. □

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After Waterman

E. Naylor

The Biology of Crustacea. Vols 1-3 of a projected ten-volume series.

Editor-in-chief Dorothy E. Bliss.

Academic: 1982/1983. Vol. 1 pp.318, \$38.50, £25.60; Vol.2 pp.440, \$49.50, £32.80; Vol.3 pp.480, \$59, £39.

A LITTLE OVER 20 years ago *The Physiology of Crustacea*, edited by T. H. Waterman, was published by Academic Press. It was a two-volume work which synthesized and stimulated the study of crustacean biology in a manner which has been of fundamental importance for the development of the subject. Now, as a measure of the success of that publication, it is argued that to produce an up-dated review under the broader title of *The Biology of Crustacea*, it is necessary to plan for ten volumes, with almost a hundred contributors and eight editors.

The past two decades have undoubtedly witnessed a remarkable growth of knowledge in many areas of crustacean biology. The study of interrelationships through comparisons of living forms has been advanced by fuller evaluation of the Class Cephalocarida, discovered only a few years before *The Physiology of Crustacea* was published in 1960, and the recent discovery of the Class Remipedia in 1980. Evidence from the fossil record has also increased markedly with new fossil finds of late Palaeozoic forms and the application of X-ray techniques to the head and limb structure of trilobites, thus clarifying their relationships with Crustacea. Significant advances have been made in the study of crustacean embryology, with evidence emerging of modified spiral cleavage and of constancy in the fate map of the blastula

which is unique among the Arthropoda.

Furthermore, studies of larval development, growth, sex determination and genetics have all pushed forward in recent years, not least in relation to the growing importance of crustaceans in aquaculture. Also, new and important studies on the neurobiology of crustaceans are concerned with the investigation of single identifiable neurons, ultrastructure and function in synapses, and the modulatory role of neurohormones on the nervous system and on behaviour.

As a reference source for these and other developments in the phylogeny, genetics, morphogenesis and neurobiology of crustaceans, the first three volumes of *The Biology of Crustacea* will no doubt be a success. These, and the later volumes in this ambitious series, are in the hands of an impressive array of authors and editors, and the whole project should contribute greatly to the further advancement of carcinology.

However, some problems of this compendium approach to book writing are already evident, and the problems seem likely to increase as later volumes appear. In some areas there is contrived compartmentalization and in others there is overlap and often conflict. Somewhat arbitrary subdivisions come through, for example in the chapter on evolution within the Crustacea (Vol. 1) which is divided into four parts (General, Copepoda, Cirripedia and Ostracoda) each written by a different author. In Vol. 2 where it is rightly argued that it is difficult to separate structure from function, the chapter on comparative morphology of crustacean appendages ignores function because that is discussed by several other authors in several other chapters in several other volumes.

The more circumscribed chapters are good and, in general, seminal, but the overlap of topics between several of the contributions often no more than reflects the controversies in the primary literature without synthesizing and developing the arguments. In Vol. 1, for example, important topics such as the origin of the Crustacea and the phylogenetic position of the Cephalocarida extend over several chapters in an uncoordinated manner. Indeed, the essence of the problem of a series of books on a topic such as this, written and edited by so many people, is crystallized in the reluctance of the editors at the outset to conclude whether the Crustacea are a Phylum, a Subphylum or a Superclass. One would like to have seen here and elsewhere the formulation of more hypotheses, albeit in the spirit of the quotation by R. W. Burkhardt referred to in Vol. 1, that "He [Lamarck] had little patience with those who did not realize that a theory could be founded upon a great number of facts and still be false". □

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Do-it-yourself in the laboratory

A. Elliott

Building Scientific Apparatus: A Practical Guide to Design and Construction.

By John H. Moore, Christopher C. Davis and Michael A. Coplan.

Addison-Wesley: 1983. Pp. 483.

\$54.95, £41.20.

THE SCOPE of *Building Scientific Apparatus* is wider than the title suggests, in that the book contains much basic theory underlying the design of physical instruments. The authors have endeavoured to provide the knowledge needed to design instruments, to communicate with the technicians in workshops or the firms who will construct them, and to inform the reader where ready-made components can be obtained. Readers in the United States will benefit most from this last feature, for American sources far outweigh all others. Similarly, inches and pounds are used as well as metric units, which reflects American practice. The text is well supported by numerous general and cited references.

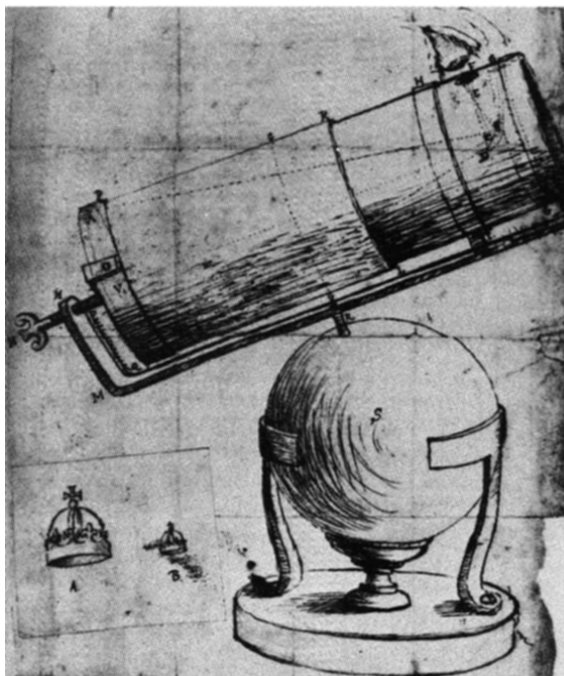
The authors start with brief accounts of mechanical design (which show the reader how to prepare working drawings) and of laboratory glass-working. The following, very useful chapter on vacuum technology adequately covers the subjects of gas flow and pressure measurement. A section on pumps includes details of sorption, getter, cryo and ion pumps, and the chapter ends with a practical section on vacuum system design and construction.

The next chapter, the longest in the book, deals with "Optics" and covers the ultraviolet, visible and infra-red regions. Here the reader is almost at once presented with the matrix formulation of optical analysis, but this is not needed to understand much of what follows and the topic would have been better relegated to an appendix. There is much useful information on optical materials, components, detectors and sources, the long section on dispersing instruments being especially good. The coverage of lasers, however, while extensive is not everywhere clear, particularly on the principles of laser operation.

Charged-particle optics is considered in the penultimate chapter, the emphasis being on electrostatic focusing devices, and the book ends with a lengthy discussion of electronics. This provides an introduction to circuit theory, which is followed by a long section on components; in addition to design, there are many details about the actual construction which are clearly illustrated by diagrams.

As to flaws, there is no treatment of X-ray optics, and little on magnets and magnetic fields, and the section on adhesives is disappointingly brief. In Table 1.3, some coefficients of thermal expansion are per °F, not per °C, as stated. For the rest, however, the book is highly recommended. The enormous progress in techniques since John Strong's *Procedures in Experimental Physics* in 1938 is indeed impressive — but one misses the delight in the home-made so evident in that book. □

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HOME-made in the seventeenth century — a reflecting telescope built by Isaac Newton in 1668–1669. When drawn to the attention of the "eminent grandees" of the Royal Society in 1671, it gained recognition for Newton who then "stepped publicly into the community of natural philosophers to which he had hitherto belonged in secret".

The illustration and quotation are taken from Richard S. Westfall's well received biography of Newton, *Never at Rest*, which has been issued in paperback by Cambridge University Press (price £12.50, \$19.95). For review see *Nature* 290, 803; 1981.

Also newly available in paperback is *The Newtonian Revolution, with Illustrations of the Transformation of Scientific Ideas* by I. Bernard Cohen. The book was reviewed in *Nature* 292, 392; 1981. Publisher is again Cambridge University Press; price is £9.95, \$16.95.

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